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STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS

XXIV. THE ALIMENTARY ABSORPTION OF CALCIUM AND ITS DEPOSITION IN THE TISSUES IN EXPERIMENTAL TUBERCULOSIS

MARY E. MAVER AND H. GIDEON WELLS

From the Otho S. A. Sprague Memorial Institute and the Department of Pathology, University of Chicago

Since calcified tubercles are usually either latent or sterile, healed tubercles, at least in man,[1] numerous therapeutic attempts have been made to stimulate calcification of tuberculous lesions with the idea that healing would thus be accomplished.[2] While we have no evidence that calcification favors rather than follows healing, it is certainly probable that a calcified tubercle is less likely to break down and liberate any remaining living tubercle bacilli than a tubercle that is not calcified. Whether administration of lime salts in any form or by any route will increase the rate of calcification is also problematical. Wersen (1) administered calcium lactate by mouth to 20 children with tuberculous peribronchial lymph nodes, and, with an equal number of untreated controls, studied them by fluoroscopy. He believed that a much larger number of the treated patients showed calcification of the nodes, as well as a greater improvement in health.

Of course, the resistance to tuberculosis of those who work in lime dust is traditional (2); but whether the calcium salts taken into the lung in these cases merely act mechanically to stimulate fibrosis, or are taken up and deposited in the tubercles, or whether they really have any beneficial influence, is not known.

The French, especially, have urged the value of calcium in tuberculosis, not so much with the idea of facilitating the calcification of tubercles, but on the hypothesis that in tuberculosis there exists a demineraliza-

[1] See Eugen Schmitz, Dissertation, Düsseldorf, 1909, and Wegelin, *Cor.-bl. f. Schweiz. Aerzte*, 1910, xl, 913.

[2] A review of the subject of the composition of calcified tuberculous lesions is given by Maver and Wells, AMER. REV. TUBERC., 1922, vi, 649.

tion, especially a loss of calcium. Robin, Ferrier, and others have written extensively on this subject, and Senator as early as 1877 stated that there was an excessive calcium excretion in the tuberculous. Sergent (3), who reviewed the early French work, was quite convinced of the value of calcium therapy. The calcium loss was supposed by some to be the result of intestinal lesions (Loeper and Béchamp (4)), while others have looked upon it as the result of a binding of the calcium by toxic products of the tuberculous infection (Croftan (5)). Most of the early clinical chemistry on which these ideas rested is inaccurate, and extensive deductions have been based upon such obviously valueless data as calcium determinations of the urine without consideration of either the calcium intake or fecal excretion of calcium. Ott (6), whose studies seem to have been more reliable than many others, found that with adequate nourishment there is no loss of calcium in phthisis; with undernourishment there is, of course, a loss of calcium as of everything else, but this is not an early feature of tuberculosis, or in any sense different from that occurring in cachexia or malnutrition from whatever cause.

A. Mayer (7) determined the balance of the inorganic elements in two severe cases of phthisis in twelve-year-old children and three adults. Although he found a marked increase in the amount of calcium excreted in the urine, this was more or less compensated by a decrease in the feces, so that in each case there was a small plus balance of calcium. There was also a lack of demonstrable evidence of loss of other inorganic elements, so that he failed to support the view that demineralization is an essential feature of tuberculosis. His general conclusions have been supported by Vannini (8) and by Labbé and Gallippe (9).

Voorhoeve (10) pointed out that the actual calcium balance in a tuberculous patient is of no significance, since decalcification is a relative term, depending on the amount of calcium supplied. He therefore studied carefully, in a few cases, the amount of calcium that was needed in the diet to maintain calcium balance, and found no very striking difference between his tuberculous and nontuberculous subjects. Certainly, if any tendency to decalification was present at all it was but little marked, but the number of cases was not sufficient to permit of drawing any conclusions. However, McCann (11) has stated that incomplete studies suggest that more calcium is required for maintenance of equilibrium in tuberculous than in normal subjects. The prevalent belief that consumptives suffer from dental caries because of abstraction of calcium could not be confirmed by Kiehnle (12).

Sarvonat and Rebattu (13), who have been advocates of calcium therapy in tuberculosis, give analyses of the tissues of four tuberculous and two normal guinea pigs, which, to quote from Kahn, they hold to prove that in the ash of the skeleton and soft tissues the content of phosphorus varies irregularly, tending to be high in the soft tissues. Their figures on the relative content of calcium of healthy and tuberculous tissues (guinea pig) are shown in the following table:

	NORMAL GUINEA PIGS	TUBERCULOUS GUINEA PIGS
	grams	*grams*
Calcium of bone	6.342	4.6
Calcium of soft parts	1.253	0.774
Relation of calcium to total (bone)	0.496	0.357
Relation of calcium to total (soft parts)	0.179	0.162

Max Kahn (14) says that he found tuberculous lung tissue twice or thrice as rich in calcium salts as normal lung, and that guinea pigs inoculated with tubercle bacilli showed a deposition of lime salts in the tuberculous foci; but no report of the details of this work have been published.

Loeper and Béchamp (15), however, found about the same amount of calcium in human lungs in tuberculosis as in other conditions, in the few cases examined by them.

Bracci (16), investigating the basis of the therapeutic use of calcium salts in tuberculosis, analyzed the lungs of rabbits to which calcium had been administered by various means, including intratracheal insufflation, and found the lung calcium always close to the normal. But rabbits that had received intratracheal insufflation of bismuth salts showed three times the normal quantity of calcium in their lungs, alike whether they were or were not given calcium. He stated his conclusions as follows:

1. The saturation of the organism of the rabbits with calcium salts in any form does not augment the amount of calcium in the lungs. 2. There exist substances in the lungs which have the property of fixing calcium in the lungs. 3. The substances which possess the property of fixing the calcium do not fix a greater quantity in the lungs of rabbits under the calcium cure. This fact agrees perfectly with the law that dominates the equilibrium of the organism. The proportion of the amount that is being assimilated is therefore invariable, and, as a matter of fact, part of the salts which are administered in excessive

quantities become eliminated without even being absorbed, part after absorption, and after their circulation through the body, are eliminated by the common ways of elimination, and part that are absorbed in the soluble form are rendered insoluble and are deposited in places where they can be gotten when needed for nutrition.

Steinitz and Weigert (17) analyzed the entire body of a year-old child, dead of tuberculosis, and found a normal total ash figure, and no abnormal distribution of the different inorganic elements of the ash, from which they inferred at least a lack of confirmation of the hypothesis that there is a demineralization in tuberculosis.

While the experiments of Tanaka and others show that injected lime salts may temporarily increase the calcium content of the blood and lead to metastatic deposition of calcium salts, yet it has not been demonstrated that feeding of these salts will modify the blood calcium in tuberculosis. Denis and Minot (18) did not find any increase of calcium in the blood of normal persons given calcium lactate by mouth, 6 gm. per day for six to ten days. Much calcium is normally excreted into the bowel, and hence it is quite probable that amounts appreciably raising the normal blood level will not be absorbed from the bowel. Nevertheless, as Givens (19) and others have found that feeding calcium salts causes an increased excretion of calcium in the urine, it seems probable that some absorption takes place in the alimentary canal, although the calcium may be so rapidly removed that it does not cause any appreciable protracted rise in the blood calcium. Thus Mason (20) found that a 5 gm. dose of $CaCl_2$ might cause a rise of plasma calcium by 2.5 mgm. per 100 cc. in three hours, but no effect was produced by calcium lactate.

Furthermore, a few observers have found that when considerable amounts of calcium are fed there may be some retention of calcium (Voorhoeve (21), Towles (22)) and increased calcium content of the tissues has been found in a few animals receiving calcium, studied by Kost (23). The accuracy of these older analytical studies may, however, well be questioned; thus, Voorhoeve used a method with an admitted error of 2.5 mgm. per 100 cc.

Howland and Marriott (24) found that when 0.5 to 1 gm. of calcium chloride was given to children with tetany, which is accompanied by a fall in the serum calcium from the normal, 10 to 11 mgm. per 100 cc. to from 4 to 7 mgm. (average 5.6 mgm.), there occurs a prompt disappearance of the tetany together with a rise of the blood calcium, some-

times to normal figures; but sometimes, despite continued calcium feeding, the serum calcium could not be sent above 7.5 to 9 mgm. Jacobowitz (25), however, was not able to increase the blood calcium by oral administration either in normal children or in those with tetany, even when the therapeutic results were excellent. Denis and Minot also observed that sometimes in animals with a low blood calcium the figure rose somewhat when calcium salts were fed, but normal figures could not be raised. Muggia (26), who could not find that there is any specific demineralization in tuberculosis, was also unable to raise the calcium content of the blood by administering inorganic calcium salts.

It is quite to be expected that figures lower than normal can be raised to normal, but that higher figures are not to be obtained, since we have it well shown by the phenomenon of metastatic calcification that the normal blood calcium represents about the maximum amount that the blood can carry, since greater amounts are promptly precipitated out in those tissues where the alkalinity is greatest or the CO_2 content the least.[3] The experiments of Heubner and Rona (27) show that even subcutaneous injection of nearly lethal doses of $CaCl_2$ (0.25 gm. per kg.) in cats produces a rise of only about 40 per cent in the blood calcium, reaching its maximum in from thirty to ninety minutes and falling to normal within five or six hours. Intravenous injections of maximal quantities may drive the blood calcium up to two or three times the normal, but the original figure is restored in about two hours, thus indicating the capacity of the body to restore the blood calcium to normal under even extreme conditions.

From the above review of the literature it is seen that *we have no experimental proof that the administration of calcium, either by mouth or otherwise, increases the amount of calcium deposited in tubercles or other necrotic areas*, unless we consider Wersen's clinical observation as experimental evidence. Nor, in view of the facts disclosed in metastatic calcification, should we expect any appreciable modification in the amount of calcium deposited in tuberculous tissues by changes in the calcium intake, except in those whose blood calcium is less than normal in amount. We know of no reliable figures by modern methods which show any deficiency in calcium in the blood in pulmonary tuberculosis. Halverson (28) and his colleagues have made careful analyses of the

[3] For a full review of the subject of metastatic calcification see Wells, *Arch. Int. Med.*, 1915, xv, 574.

blood in patients with pulmonary tuberculosis, and draw the following conclusions:

> Determination of the calcium content of the blood of patients with advancing and convalescing tuberculosis revealed that in the incipient cases in which the patients, who were on a high milk diet, showed marked improvement, the values for calcium in the serum were normal and fairly constant. In no case was the calcium value increased above normal figures by this high calcium diet. In advanced cases the variations were greater (some rather high and some rather low values being obtained), and improving patients showed on the average slightly higher values than the unimproved. No marked deviations from the normal, however, were observed in the calcium content of the serum of patients in the various stages of pulmonary tuberculosis.
>
> The failure of the body to deposit lime around the tuberculous areas is to be ascribed, not to a deficiency in blood calcium but rather to an inability of the cells of the tuberculous area properly to utilize available calcium. The total blood of a 60 kg. man contains about 0.3 gm. of calcium. The bones contain approximately 4,000 times as much. With this great reserve supply of calcium, a portion of which is available, as is indicated by the great constancy of blood calcium under varying diets and conditions, a physiological basis for the administration of calcium salts in tuberculosis has yet to be demonstrated.

While this last argument, as to the futility of giving calcium by mouth while the bones contain great quantities of calcium, is striking, it is not wholly convincing in the face of the existence of such conditions as tetany and some forms of nephritis, with a reduced blood calcium which can be restored to normal by giving calcium salts by mouth, with a coincident amelioration of the symptoms of calcium deficiency. Apparently, the bone salts are not always available for general metabolism.

In view of all this evidence, therefore, it seems *a priori* altogether doubtful that calcification of tuberculous lesions can be appreciably altered by oral administration of calcium salts. Whether a tubercle undergoes calcification or not would seem to depend chiefly, if not solely, upon how long it remains as a quiescent mass of colloidal organic material, presenting such physical properties as favor the deposition of calcium salts (29); that is, a human tubercle is probably at least latent before it begins to calcify. Furthermore, tuberculous lesions in cattle show partial calcification at all stages, yet seem to progress unimpeded by this process, and certainly bone tissue itself seems to be anything but resistant to tuberculosis.

It is, of course, perfectly possible that calcium is of value in the growth of connective tissue as silica is said to be, but we lack any proof of a shortage of calcium in tuberculous tissues, or of a greater capacity of connective tissues to grow in calcium-treated animals. Furthermore, the usual large daily intake of milk in the diet of the tuberculous patient guarantees an abundance of calcium.

Numerous clinical reports of favorable results from the use of calcium have been published, and are entitled to consideration, at least on the basis of their number. However, if there has ever been published an adequately controlled clinical study of the value of calcium therapy in tuberculosis we have not succeeded in finding it.

Although occasional writers on the clinical use of calcium refer vaguely to experiments which they have performed, specific reports are few. Van Gieson and Lynah (30) (31) have published enthusiastic approval of Russell's method of treatment (32), which is based on the doubtful theory that administration of hydrochloric acid to patients increases the amount of gastric rennin, which causes calcium to combine in an assimilable form with casein and have supported it only with observations on three dogs. Two of these dogs on a calcium-poor diet showed more tuberculous lesions than one on a normal diet.

Emmerich and Loew (33) call attention to Hamburger's demonstration that calcium salts make the phagocytes more active, and report experiments in which the resistance of guinea pigs to anthrax seemed to be increased by calcium feeding. Mice were also made more resistant against hog cholera by feeding calcium. Their experiments on tuberculosis consisted in inoculating 22 guinea pigs with tubercle bacilli of unspecified origin or virulence. Twelve of these had been fed 0.1 gm. per kilo of calcium chloride, daily, for from five to eleven months. The inoculation took place on the 18th of May and by the 27th of October two of the control and four of the calcium animals were alive, but all died before the 19th of November. The calcium guinea pigs in general held their weight better and showed somewhat less tendency to the presence of pulmonary lesions. As will be seen, the difference obtained was only one of degree and this was not constant. These authors speak also of the clinical use of calcium, mentioning a favorable influence on digestion exerted by calcium chloride, but the statements as to clinical results are very vague.

Massini (34) injected 10 rabbits with bovine tubercle bacilli and gave 1 cc. of 1 per cent $CaCl_2$ solution intravenously twice a week to half of

them. As these treated animals lived an average of six days longer than the other five he looks upon this as a "small success," although the range in the treated animals was from 75 to 120 days, and in the untreated from 68 to 116 days. It is difficult to interpret these figures as even a "small success." Michelazzi (35) infected 30 rabbits and guinea pigs, and found calcification of the lesions in rabbits treated with calcium, and in the guinea pigs a cirrhosis of the liver in the treated animals marked an increased resistance. As the calcified lesions in the rabbits were sterile he believed that he had obtained a curative effect. This work has not, as far as we can find, been repeated and confirmed.

Laitinen (36) reports two series of experiments (each on 20 to 30 guinea pigs), in which the animals fed calcium phosphate lived twice as long as the controls, but calcium hydroxide had no influence. Obviously, the duration of life in so small a number of animals as this has little significance in view of the great variations seen in any set of infected guinea pigs.

On the other hand, Leo (37) and Finsterwalder (38) observed no influence of calcium treatment on tuberculous guinea pigs. Kindborg found that calcium salts favor growth of tubercle bacilli in culture media. This was corroborated by Takenaka (39), who found that the calcium ion itself has *in vitro* no inhibiting action on the tubercle bacilli but rather favors growth, although some calcium salts are inhibitory. Of these he recommends trial of calcium salicylate, which causes an increase of both red and white cells when injected intravenously into rabbits. Intravenous injections of calcium chloride into four rabbits produced in three a diuresis with nitrogen retention, which he thinks may be a favorable factor in tuberculosis therapy.

Another way in which calcium may possibly be of influence is through its action on the leucocytes. Hamburger found that calcium ions have a strikingly positive chemotactic effect on leucocytes, which has been corroborated by Miss Wolf (40), but just how an increase in the calcium content of either the blood or the tissues as a whole could affect the action of leucocytes in a tuberculous lesion is not clear. Neither can we imagine an influence from calcium on the basis of the transitory rise in the proportion of polymorphonuclear leucocytes which follows injection of calcium salts (Rösler (41)). A general favorable effect of calcium on tissue metabolism has also been suggested.[4] However, the statistical

[4] See Starkenstein, *Therap. Halbmonatschr.*, 1921, xxxv, 553.

study of the influence of hardness of drinking water by Opitz (42) did not disclose any more favorable tuberculosis rates in populations drinking water containing much lime.

It must be freely admitted that when the experience of large numbers of clinicians, familiar with the course of tuberculosis in man, endorses the use of calcium as giving definite clinical benefit, we must listen with respect to this endorsement, even if we cannot explain such results or reproduce them in experimental animals. The resistance of workers in lime dust to tuberculosis is a world-wide belief,[5] and hence in all probability not groundless. On the other hand, the fact that most standard works on tuberculosis say little or nothing concerning calcium as an agent of value in the treatment of this disease would indicate that the enthusiastic opinions of the few have not been corroborated by the experience of the many.[6] Neither are negative clinical results lacking.[7]

A review of the literature by Max Kahn (43) concludes with this statement, "In looking over the mass of literature on this subject, one is left in doubt whether the use of lime in the treatment of tuberculosis is to be recommended."

Nevertheless, in view of the supposed clinical benefits, and especially the results reported by Wersen on the calcification of tuberculous lymph nodes under calcium therapy, it has seemed desirable to study experimentally the influence of calcium feeding upon the deposition of calcium in tuberculous and nontuberculous tissues under conditions that can be controlled.

THE CALCIUM CONTENT OF THE TISSUES OF NORMAL ANIMALS

The first analyses, for control, were made of the tissues of normal guinea pigs on the ordinary laboratory diet (oats, hay and carrots) and of normal guinea pigs given every day (except Sundays) each 0.188 gm. calcium lactate (containing 0.0338 gm. Ca) by mouth in gelatin capsules.

[5] See review by Tweddell, *Med. Rec.*, 1922, ci, 141.

[6] The use of calcium to check diarrhoea, as recommended by Maendl (*Ztschr. f. Tuberk.*, 1917, xxviii, 334; also *Med. Klin.*, 1920, xvi, 228), Ringer and Minor, AMER. REV. TUBERC., 1922, v, 876, and others, is not based on any action on the tubercle bacillus itself, or apparently on the tuberculous lesions, but depends on the effect of calcium on exudative processes, and is analogous to the use of calcium to increase coagulation in hemoptysis (Maendl, *Ztschr. f. Tuberk.*, 1921, xxxv, 184). This form of calcium therapy is not related to the use of calcium for a definite effect on the tuberculosis and is outside the theme of our discussion.

[7] See, for example, Petheö, who observed no benefit in 240 cases of juvenile tuberculosis. (Reference in *Zentralbl. f. Tuberk.*, 1922, xvii, 317.)

This dose is equivalent to one of 25 to 30 gm. calcium lactate daily to an adult human being.

Methods: Immediately after autopsy the tissues removed for analysis were dried of excess blood by wiping, and put into 95 per cent alcohol until analyzed, except for portions kept for microscopic study. For analysis they were removed from alcohol, cut into fine pieces, and dried to a constant weight at 110°C. It had been intended to analyze separately the tuberculous lesions and the nontuberculous portions of the same organs, but this was found to be possible in only a few cases. Since the amount of fat varies so much in the liver, this organ was extracted with ether in a Soxhlet extractor for six hours to remove this source of error. The bones analyzed were in each case a tibia and a fibula, which were washed in cold water and air-dried.

The tissues were completely ashed, and the ash dissolved in 0.5 N hydrochloric acid. The solution was filtered from possible minute carbon particles through a small ash-free filter paper (Whatman No. 30) into a 50 cc. centrifuge tube. Concentrated NH_4OH was added, drop by drop until the reaction was very slightly alkaline to phenolsulphonephthalein. The method described by Kramer and Tisdall (44) for the determination of calcium and magnesium in small amounts of serum or plasma, was applied with slight modifications to this solution. One drop of 0.1 N H_2SO_4, 2 cc. of saturated ammonium oxalate and 0.5 cc. saturated solution of sodium acetate were added, and the mixture was allowed to stand at least one hour. The volume of the mixture was made up to 8 cc., and the pH was about 5.4, an acidity which prevents the precipitation of calcium phosphate. The mixture was centrifugated for twenty minutes and washed with 2 per cent ammonium hydroxide. The washing and centrifugating were repeated twice. The precipitate was dissolved in 2 cc. 0.1 N sulphuric acid, heated on a boiling water bath, and titrated with 0.01 N $KMnO_4$, using a micro-burrette. (The large amounts of calcium in the bones permitted gross quantitative determinations.)

The results of these analyses are given in tables 1 and 2.

It will be seen that, while in the normal animals there is not a little variation in the calcium content of the same tissues in different animals and in the different tissues in the same animal, yet averaged together the amounts are not far from the same, ranging from 38 mgm. Ca per 100 gm. of liver to 52.8 mgm. Ca in 100 gm. of lymph nodes. The fact that lymph nodes generally contain more calcium than the other tissues probably depends on the inhalation of calcium dust, which will be particularly abundant in the cages of animals fed calcium, and its deposition

in the peribronchial and cervical lymph nodes.[8] If we consider that the tissues normally contain about 75 per cent of their weight as water, it will be seen that they contain about 10 to 13 mgm. of calcium per 100 gm. moist weight, or quite the same as the blood itself. Therefore, it

TABLE 1

Calcium determinations of organs of normal guinea pigs

NUMBER	WEIGHT OF ANIMAL	PERCENTAGE OF CALCIUM IN BONES	CALCIUM IN 100 GMS. DRIED TISSUE						
			Heart	Kidneys	Muscle	Liver	Lymph nodes	Lungs	Spleen
	grams		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	518	20.1	52	21	40	32		51	
2	450	21.8	65	43	48	29	71		38
3	625	23.7	51	53	49	47		43	80
4	575	23.45	47	19	54	40		40	38
5	490	22.34	35	44	36	37	55	30.3	
6	485	24.94	71	48	37	30	25	56	61
7	570	24.98	44	64	57	26	50	65	47
8	470	20.33	43	32	46	28	62	65	20
9	480	24.08	54	58	54	22	65	73	45
10	420	26.67	30.8	33.8	35		60	34	62
11	480		69	16	42	67	71	55.7	58
12	410		52	65	40	60	37	32	46
Average		23.09	51.15	41.40	44.6	38.00	52.8	48.5	49.5

TABLE 2

Calcium determinations of organs of normal guinea pigs fed 0.0338 gm. calcium daily

NUMBER	NUMBER OF DAYS FED CALCIUM	PERCENTAGE OF CALCIUM IN BONES	HEART—MGM. CA. PER 100 GM. DRIED TISSUE	KIDNEYS—MGM. CA. PER 100 GM. DRIED TISSUE	MUSCLE—MGM. CA. PER 100 GM. DRIED TISSUE	LIVER—MGM. CA. PER 100 GM.	LYMPH NODES—MGM. CA. PER 100 GM.	LUNGS—MGM. CA. PER 100 GM.	SPLEEN—MGM. CA. PER 100 GM. DRIED TISSUE
36	15	21.92	58.24	37.8	23.7	53.8	64.5	58.6	83.2
31	27	21.78	83.02	58.5	20.0	35.7	63.0	42.4	47.3
32	41	26.86	103.01	65.3	32.1	17.1	41.7	41.0	41.0
33	56	25.15	61.1	92.0	11.7	47.6	56.7	28.0	44.6
34	87	22.42	46.9	71.6	25.4	17.0	52.0	43.0	64.1
35	95	24.40	46.2	62.6	17.8	39.4	87.2	71.3	47.1
15	182	27.96	79.5	41.5	19.8	36.7	78.2	41.7	70.2
14	213	21.02	63.2	41.0	10.7	23.1	292.8	62.7	40.7
13	214	18.67	64.7	38.5	34.2	56.04	113.0	58.0	36.7
16	228	25.84	40.5	26.8	48.2	38.0			49.3
Average		23.60	64.63	53.56	24.36	36.44	94.3	49.6	52.43

[8] See Maver and Wells, *The Chemical Composition of Calcified Tuberculous Lesions*, AMER. REV. TUBERC., 1922, vi, 649.

is apparent that the calcium is in equilibrium between the blood and the tissues.

For comparison we have in table 2 the results of analyses of the tissues of ten normal guinea pigs fed calcium for varying periods of time (15 to 228 days). Here we find that the bones, liver, lungs and spleen contain about the same amount of calcium as the unfed controls, the muscles contain less, and the kidneys, lymph nodes and heart contain more calcium. Since the kidney excretes calcium, it is to be expected that its calcium content will be raised by the increased amount of calcium in the urine in the renal tubules, and perhaps in passage through the renal tubules. It has been found by Wells and Hedenburg (45) that ingested iodides are also found in the kidneys in larger amounts than in any other normal tissue. The high figure for the lymph nodes probably depends on deposition of inhaled calcium dust, as mentioned previously, for except for two nodes with very large amounts of calcium the lymph nodes of the calcium-fed guinea pigs contained but little more calcium (62 mgm. per 100 gm.) than in the control guinea pigs. The high figure for the heart depends on one exceptionally high result, which gives rise to the suspicion that this heart may have contained some calcified lesion not noticed at autopsy. Similar instances of occasional high calcium content in the heart have been found in other series of experiments. Disregarding these exceptional instances, we find that administration of calcium to normal guinea pigs has not generally increased appreciably the calcium content of their tissues. No recognizable difference is found between animals that have received calcium for long or for short periods, indicating that in normal animals, at least, there is no tendency for administered calcium to accumulate in the tissues.

In order to secure evidence as to whether there is any retention of calcium in the body as a whole, perhaps not exhibited by the organs and tissues analyzed in these guinea pigs, two series of experiments were carried out with mice, since these animals can readily be analyzed entire.

Twelve mice were fed a mixture of one part whole milk powder (*Dryco* = fat, 12 per cent; lactose, 44 per cent; protein, 34 per cent; salts, 7 per cent; moisture, 3 per cent) and two parts yellow corn meal, being permitted to eat as much as they would.

Twelve mice were fed the same mixture, containing in addition 5 per cent of calcium lactate. The mice were fed this diet for twelve days. On the evening (eight o'clock) of the twelfth day all food was

taken from them and they were starved until nine o'clock of the following day. They were then killed with illuminating gas, skinned, since there might well be adherent dust, and their alimentary tracts removed. The bodies were then weighed without drying.

There was evidence of diarrhoea among both sets of mice, but at autopsy the evidence was much more marked in the calcium-fed mice. The results are given in table 3.

It is seen that the total amount of calcium in the entire ash of the two sets of mice is practically the same (0.1643 gm. as aganst 0.1588 gm.), there being a trifle more in the mice *not* fed calcium. The percentage of calcium in the total fresh weight is, however, a little higher

TABLE 3

MICE NOT FED CALCIUM				MICE FED CALCIUM			
Number	Grams fresh weight of skinned mouse	Grams total calcium in ash	Percentage of calcium	Number	Grams fresh weight of skinned mouse	Grams total calcium in ash	Percentage of calcium
1	9.1668	0.1470	1.60	13	10.2486	0.1443	1.40
2	13.1661	0.1714	1.32	14	10.0837	0.1504	1.49
3	14.2815	0.1647	1.15	15	10.1253	0.1762	1.74
4	13.9001	0.1720	1.23	16	12.0577	0.1551	1.23
6	14.4465	0.1767	1.22	17	12.2373	0.1744	1.42
7	14.2364	0.1668	1.17	18	12.1554	0.1925	1.58
8	13.7274	0.1580	1.15	19	11.2468	0.1946	1.73
9	11.3528	0.1443	1.27	20	8.2940	0.1580	1.90
10	13.7592	0.1615	1.17	21	9.2034	0.0972	1.05
11	11.9963	0.1727	1.40	22	12.4045	0.1685	1.36
12	14.8981	0.1729	1.16	23	10.2681	0.1363	1.32
Averages	13.1755	0.1643	1.25		10.7568	0.1588	1.47

in the mice that were fed calcium (1.25 per cent and 1.47 per cent), which probably depends on their being more dehydrated by the diarrhoea, as indicated by the relatively low weight of these mice.

Because of the complicating influence of the diarrhoea, this experiment was repeated, the diarrhoea being prevented by adding filter paper fragments to the feed. The analytical results are given in table 4.

In this series there was little or no diarrhoea, and here the total calcium in the mice of each series is practically identical (average, 0.1441 and 0.1449 gm.), the percentage of calcium being a trifle higher in the mice not fed calcium, but not beyond the difference possible from the chances of sampling in a series no larger than this.

These experiments corroborate the observations on the guinea pigs in indicating that the administration of relatively large amounts of calcium salts to animals on a normal diet does not increase the amount of calcium in their bodies. Under these conditions the extra calcium is not at all retained.

TABLE 4

MICE NOT FED CALCIUM				MICE FED CALCIUM			
Number	Grams weight of mouse	Grams total calcium	Percentage of calcium	Number	Grams weight of mouse	Grams total calcium	Percentage of calcium
11	12.5365	0.1360	1.09	1	11.4432	0.1284	1.12
12	11.5709	0.1642	1.43	2	12.4160	0.1651	1.32
13	11.0702	0.1482	1.33	3	9.7712	0.1114	1.14
14	8.9281	0.1182	1.31	4	14.2517	0.1565	1.09
15	11.7241	0.1366	1.16	5	12.0084	0.1441	1.18
16	10.5983	0.1579	1.45	6	15.1850	0.1855	1.15
17	9.7325	0.1252	1.31	7	10.3635	0.1459	1.40
18	13.8150	0.1667	1.20	8	12.2848	0.1650	1.34
				9	11.5637	0.1393	1.26
				10	11.7688	0.1280	1.09
Averages	10.4969	0.1441	1.28		12.1056	0.1449	1.209

THE CALCIUM CONTENT OF THE TISSUES OF TUBERCULOUS ANIMALS

We next undertook to ascertain the influence of tuberculosis on the calcium content of the tissues, and, if possible, the influence of calcium administration on the tuberculosis. For this purpose the following experiments were carried out:

Series 1: Twelve guinea pigs, weighing from 508 to 638 gm., were given each a subcutaneous injection of 0.5 mgm. of a strain of human tubercle bacilli of low virulence[9] (no. 1305), on January 31, 1921. One month later the regional lymph nodes were palpable, and on March 3rd 6 of the animals were selected and each given from that time daily, except Sundays, 0.188 gm. calcium lactate (containing 0.0338 gm. Ca) by mouth in gelatin capsules. At the same time 6 normal guinea pigs were started on the same course of calcium feeding. Of this series of 18 animals, 2 died early of pneumonia and a third was discarded as it bore young, leaving five sets of 3 animals each; that is, (1) tuberculosis plus calcium, (2) tuberculosis without calcium and (3) calcium without

[9] We wish to express our obligation to Dr. Lydia M. DeWitt, for the inoculation of the animals used in these experiments.

tuberculosis. All were kept under the same conditions and on the ordinary laboratory diet of oats, hay and carrots. When one of these guinea pigs died, the other two of the set were killed, and the tissues analyzed for calcium.

None of the animals of this series died of tuberculosis, because of the low virulence of the strain of bacillus used. Six of ten showed evidence of tuberculous infection, in the form of enlarged lymph nodes at the site of inoculation, and in two there was a slightly enlarged spleen. One (no. 9) died of pneumonia seventy-four days after inoculation, and one normal calcium-fed guinea pig (no. 18) died of pericarditis and pneumonia. The analytical results obtained with these animals are given in tables 5 and 6 and show no particular differences between the animals which

TABLE 5

Calcium determinations of organs of guinea pigs with chronic tuberculosis and not fed calcium

NUMBER	NUMBER DAYS LIVED AFTER INJECTION	PERCENTAGE OF CALCIUM IN BONES	CALCIUM PER 100 GMS. DRIED TISSUE						
			Heart	Kidneys	Muscle	Liver	Lymph nodes	Lung	Spleen
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
4	107	29.32	90.0	53.0	35.9	50.0	48.4	37.5	270.0
3	178	26.11	76.7	43.2	32.4	26.82	252.6	65.4	33.5
2	213	26.75	53.7	40.0	36.1	35.6	98.2	42.2	86.2
5	213	25.84	42.3	59.8	24.9	37.7	54.3	58.5	50.7
6	213	26.67	55.4	73.5	15.4	22.4	40.5	63.4	53.0

Nos. 2 and 6 were very well nourished, and apparently entirely normal. Nos. 3 and 5 had slightly enlarged inguinal lymph nodes. No. 4 had enlarged lymph nodes. Its spleen was not enlarged but showed nodular caseous tuberculosis. Its lungs were apparently normal.

received calcium and the controls. It is to be noted that the only spleen which showed much tuberculosis, that of no. 4, was the spleen with the greatest amount of calcium, and was from an animal which had not been given calcium.

Series 2: Twelve guinea pigs, weighing from 337 to 555 gm., were injected subcutaneously May 4, 1921, with 0.25 mgm. of no. 4015, a highly virulent culture of human tubercle bacilli. Six of these animals died of an acute intercurrent infection, because of which, on May 25th 13 more were given a similar inoculation. On June 8th the local lymph nodes were palpable, and calcium feeding was started as in *series 1* (0.0338 gm. Ca daily) with 6 of these animals.

All animals of this series showed evidences of tuberculous infection at autopsy. Six of them died of tuberculosis (nos. 49, 42, 40, 39, 37,

19), two were killed as they were dying (nos. 38 and 42), while 9 were killed as controls to those that died (nos. *20*, *21*, 28, 30, *43*, *44*, *46*, *47*, *48*). The numbers in italics are those of the guinea pigs that had received calcium, and it will be noted that these in general outlived the animals that had not received calcium. Two animals died of undetermined cause, not tuberculosis (*45* and 41).

The details of these analyses are given in tables 7, 8 and 9.

Consideration of tables 5 to 9 for the tuberculous animals shows that while the organs which do not have tuberculous lesions (heart, kidneys, muscle) contain much the same quantity of calcium as do the same organs of nontuberculous animals, yet in the organs showing the most

TABLE 6

Calcium determinations of organs of guinea pigs with chronic tuberculosis and fed 0.0338 gm. calcium daily

NUMBER	NUMBER DAYS LIVED AFTER INJECTION	NUMBER DAYS FED CALCIUM	PERCENTAGE OF CALCIUM IN BONES	CALCIUM PER 100 GMS. DRIED TISSUE						
				Heart	Kidneys	Muscle	Liver	Lymph nodes	Lung	Spleen
				mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
9	107	74	22.91	49.0	31.0	86.0	46.0	240.0	54.0	68.0
10	178	147	28.17	67.6	46.9	44.3	18.7	68.0	61.1	51.04
11	213	182	23.09	35.4	40.1	28.1	32.1	7.0	50.8	64.5
12	213	182	26.07	63.6	76.8	54.5	30.0	108.9	51.4	19.5
7	213	182	27.04	55.8	59.07	40.0	23.3	20.7	57.5	82.6

No. 9 died of pneumonia: its spleen and lymph nodes appeared normal. Nos. 10 and 11 were killed: their lymph nodes at the site of inoculation were slightly enlarged. No. 12 was killed: on microscopic examination of the spleen numerous typical tubercles of the epithelioid type were seen: the lymph nodes showed extensive fibroplastic tuberculosis. No. 7 was killed: it was apparently normal throughout.

marked lesions (spleen and lymph nodes) the proportion of calcium is greatly increased. This illustrates the tendency of calcium to deposit even in actively progressing tuberculous lesions,[10] but it is to be noted that the amount of calcium in the tuberculous spleens and lymph nodes may be either less or greater in the animals that did not get calcium.

The bones of tuberculous animals, whether fed calcium or not, contain normal quantities of calcium, a finding which fails to support the statement of Sarvonat and Rebattu (based on six animals), that there is a depletion of calcium in the bones.

[10] Caldwell (*J. Infect. Dis.*, 1919, xxiv, 81) found that bovine tuberculous tissues which do not exhibit any macroscopic evidence of calcification often have a very high calcium content.

TABLE 7

Calcium determinations of organs of acutely tuberculous guinea pigs which were not fed calcium

NUMBER	NUMBER DAYS LIVED AFTER INJECTION	PERCENTAGE OF CALCIUM IN BONES	CALCIUM IN 100 GMS. DRIED TISSUE						
			Heart	Kidney	Muscle	Liver	Lymph nodes	Lung	Spleen
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
40	54	23.55	61.6	67.1	61.8	25.6	56.2	31.4	39.2
28	55	23.35	113.2	85.6	55.4	160.0	106.2	37.0	137.9
41	65	24.75	55.1	63.3	64.4	20.6	78.2	40.5	143.1
37	65	25.87	54.7	63.7	46.4	20.6	125.5	73.6	673.0*
38	78	24.43	62.9	61.8	12.1	52.8	104.9	37.4	64.9
30	100	25.80	51.4	58.1	36.8		150.9	48.4	39.7
49	105	26.90	76.2	81.5	13.8	7.4	19.0	29.2	3.5
24	107	23.16	44.9	85.5	28.2	24.6	125.9	69.3	26.13
42	131	25.84	77.2	53.9	52.8	16.6	159.08	94.1	119.0*
39	144	25.32	56.9	26.4	9.8	23.1	181.0	129.7	39.9
Averages		24.89	65.41	64.69	38.15	46.8	100.58	59.06	128.6

* The tuberculous portions of the spleens of nos. 37 and 42 were analyzed separately from the nontuberculous portions. Calcium estimations were: no. 37, tuberculous portion, 2027.8 mgm., nontuberculous portion, 250.6 mgm.; no. 42, tuberculous portion, 143.6 mgm., nontuberculous portion, 59.0 mgm.

No. 40: Died of tuberculous pneumonia. Histological examination showed a few tubercles in lung; in spleen many active miliary tubercles, comprising about 30 per cent of tissue of spleen. The lymph nodes moderately enlarged.

No. 28: Killed as control. Histological examination of liver revealed numerous tubercles; the lung numerous typical tubercles; the spleen had more tubercles than normal tissue. A parapancreatic lymph node contained large tubercles and small focal tubercles. Macroscopically, the lymph nodes were much enlarged, and some were caseous.

No. 41: Died. Spleen studded with miliary tubercles. Histologically, lungs showed no tubercles, but some edema. The liver was considerably enlarged and contained a few superficial necrotic areas. Lymph nodes only slightly enlarged.

No. 37: Died. Histological examination of the lungs showed that over 50 per cent had been replaced by tuberculous tissue. In the liver there was much fibrosis, as in repair of recent necrotic areas, but there was no distinct tuberculosis. The spleen contained numerous tubercles and some necrotic areas. The lymph glands were largely tuberculous.

No. 38: Killed as control. Histological examination. The lung tissue was largely replaced by consolidated areas, not definitely tuberculous. In the spleen, which was enlarged, there were no distinct tubercles. The liver had necrotic areas, but no tubercles.

No. 30: Killed as control. Histological examination: In the spleen and liver, a very few miliary tubercles. The lungs contained very many tubercles and much edema. A peritracheal lymph node was largely replaced by tuberculosis. The inguinal and peribronchial lymph nodes were much enlarged and caseous.

No. 49: Died. Histological examination of the liver showed it to be fatty, but no tubercles were seen. In the lungs there were many miliary tubercles, amounting to about 10 per cent of the lung tissue. The spleen was greatly congested with some necrosis, but no typical tubercles were seen. One inguinal lymph node was slightly enlarged and caseous.

No. 24: Killed as it was dying. The lymph nodes in inguinal region were much enlarged and caseous. Histologically, the spleen was seen to be riddled with active tubercles, comprising about 25 per cent of the spleen tissue. In the lungs there were occasional active tubercles. The liver was apparently normal.

No. 42: Died. The inguinal and peribronchial lymph nodes were enlarged and caseous. Histological examination of the spleen showed that it was largely necrotic, with areas resembling infarcts, which were not typical tubercles. The lungs showed numerous large acute tubercles. The liver contained many areas of fibrosis and only a few active tubercles.

No. 39: Died. Histological examination of a lymph node showed many small, rather fibrous tubercles, and large necrotic areas, some of which were distinctly calcified. The liver contained much connective tissue formation and fatty change, but no tubercles. The lung showed large masses of consolidation resembling infarcts, probably tuberculosis. The spleen contained many acute tubercles.

TABLE 8

Calcium determinations on organs of acutely tuberculous guinea pigs which were fed calcium

NUMBER	NUMBER DAYS LIVED AFTER INJECTION	NUMBER DAYS FED CALCIUM	PERCENTAGE OF CALCIUM IN BONES	CALCIUM IN 100 GMS. DRIED TISSUE						
				Heart	Kidneys	Muscle	Liver	Lymph nodes	Lung	Spleen
				mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
47	54	41	24.32	67.0	60.5	14.0	39.2	32.0	91.3	91.6
19	55	20	15.27	80.3	6.0	88.8	38.0	1298.0	60.1	22.1
48	65	50	23.26	49.8	81.6	37.4	33.0	67.6	38.1	50.8
45	69	19	23.19	160.2	36.2	67.3	317.8	301.0	80.9	136.0
46	78	65	25.52	62.5	71.4	37.4	19.6	47.0	60.4	104.4
20	100	64	26.36	86.4	80.0	48.1	70.7	257.8	51.7	190.5
43	131	87	25.65	86.4	58.9	65.00	22.8	132.6	27.0	26.0
21	133	95	25.80	67.7	53.1	37.07	20.3	111.4	38.3	278.0
44	144	131	24.82	44.4	47.5	20.5	16.0	73.3	43.2	61.7
Averages.....			23.78	78.3	55.02	46.17	64.15	257.8	54.5	106.78

No. 47: Killed as control. The inguinal and peribronchial nodes were enlarged, and some were caseous. On histological examination it was found that about 50 per cent of the spleen was made up of tubercles. The lungs showed focal fibrous areas, with occasional distinct tubercles. No tubercles were seen in the liver.

No. 19: Died of pneumonia. The lymph nodes were very much enlarged and caseous. Microscopic examination of the spleen showed that about 60 per cent of it was replaced by epithelioid tubercles. The liver had infarcted areas, some necrotic areas and some fatty change. The lungs had large pneumonic areas, not definitely tuberculous.

No. 48: Killed as control. The inguinal and peribronchial nodes were enlarged. Microscopically, 25 per cent of the spleen was replaced by many small tubercles. The liver contained a few tubercles with a moderate degree of perilobular fibrosis. The lungs had a very few miliary tubercles.

No. 45: Died. The lymph nodes were only slightly enlarged and caseous. The spleen was studded with miliary tubercles. The liver showed a few small necrotic areas only. Lungs showed no visible tuberculosis. Postmortem degeneration too far advanced to tell cause of death.

No. 46: The lymph node in left inguinal region, the site of inoculation, was slightly enlarged and caseous. Microscopically, this node was seen to have occasional epithelioid tubercles. The spleen and liver were apparently normal. The lungs had occasional small tubercles.

No. 20: Killed. The inguinal and peribronchial nodes were much enlarged. They showed, microscopically, much fibrosis with a few active tubercles. The spleen was only moderately enlarged, but covered with nodular tubercles. The liver, microscopically, had a few fibrous tubercles. The lung had numerous tubercles, mostly fibrous.

No. 43: Killed. The peribronchial lymph nodes were slightly enlarged. Microscopic examination of the lungs showed numerous tubercles. The spleen had numerous small active tubercles. The liver showed occasional small tubercles, and much fatty change.

No. 21: Killed. The lymph nodes in the inguinal and peribronchial regions were decidedly enlarged and caseous. The spleen was much enlarged and covered with numerous tubercles. The liver had a few small tubercles. There were a few tubercles visible in the lungs.

No. 44: Killed. At autopsy the only evidences of tuberculosis consisted of one enlarged caseous lymph node at the site of injection and much enlarged peribronchial nodes. Microscopically, these nodes showed extensive fibroplastic tuberculosis. The lungs were edematous but no tubercles were seen.

For some reason the lung, even when extensively tuberculous, is much less likely to show a high calcium figure than either the liver or the spleen. It is to be noted, if the anatomic findings are compared with the chemical findings, that there are many exceptions to the rule that the more tuberculosis an organ shows the more calcium it contains.

The calcium determinations of the lymph nodes and spleens of 18 tuberculous animals, regardless of presence of gross lesions in the spleen (9 fed calcium and 9 not fed calcium), show that in 5 out of 9 pairs of animals, the calcium content of the lymph nodes of the calcium-fed guinea pigs was less than that of the guinea pigs which were not fed calcium. In 5 out of 9 pairs of animals the calcium content of the spleens of the calcium-fed guinea pigs was greater than that of the animals not fed calcium.

However, the extent of the tuberculous lesions was much greater in the lymph nodes in 7 out of 9 cases in the control animals, and in the spleens, in every case, the extent of the tuberculosis was much greater in the control than in the calcium-fed guinea pigs. The average calcium content of the lymph nodes of the calcium-fed animals was 257.8 mgm. per 100 gm. dried tissue, as against 100.58 in the guinea pigs not fed calcium, and the average calcium content of the spleens of the calcium-fed pigs was 106.78 mgm. as against 128.6 in those not fed calcium.

It is somewhat difficult to evaluate these average figures, however, since they are made up of single estimations with such great variations. Compare, for example, spleen no. 37 with an average of 673 mgm. cal-

TABLE 9

General summary of acutely tuberculous guinea pigs

	PERCENTAGE OF CALCIUM IN BONES	CALCIUM IN 100 GMS. DRIED TISSUE						
		Heart	Kidneys	Muscle	Liver	Lymph nodes	Lungs	Spleen
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Averages for all (20) calcium-fed guinea pigs, with or without tuberculosis	23.32	71.10	54.25	34.69	55.86	176.05	52.06	78.18
Min.-Max.	15.27–27.96	40.5–160.0	6.0–92.0	10.7–88.0	16.0–317.8	32.0–1298	27.0–91.3	22.1–278.0
Average for (9) calcium-fed tuberculous guinea pigs	23.78	78.3	55.02	46.17	64.15	257.8	54.5	106.78
Min.-Max.	15.27–26.36	44.0–160.0	6.0–81.6	14.0–88.0	16.0–317.8	32.0–1298	27.0–91.3	22.1–278.0
Average for (11) calcium-fed guinea pigs, not tuberculous	23.85	64.57	53.56	24.33	36.44	94.3	49.6	52.43
Min.-Max.	18.67–27.96	40.5–103.0	26.8–92.0	10.7–48.2	16.0–317.8	32.0–1298.0	27.0–91.3	22.1–278.0
Average for (10) tuberculous guinea pigs, not fed calcium	24.89	65.41	64.69	38.15	46.8	100.58	59.06	128.6
Min.-Max.	23.16–26.90	44.9–113.2	26.4–85.6	9.8–64.4	7.4–160.0	19.0–181.0	29.2–129.7	3.5–673.0
Average for (12) guinea pigs not fed calcium and not tuberculous	23.09	51.15	41.40	44.6	38.00	52.8	48.5	49.5
Min.-Max.	20.1–26.67	30.8–71.0	16.0–65.0	35.0–57.0	26.0–67.0	25.0–71.0	30.0–73.0	20.0–80.0

TABLE 10

Relation of calcium deposition to tuberculous lesions in acutely tuberculous guinea pigs

	CALCIUM IN 100 GMS. DRIED TISSUE			
	Liver	Lymph nodes	Lungs	Spleen
	mgm.	*mgm.*	*mgm.*	*mgm.*
Averages for all tuberculous guinea pigs	55.4	175.05	56.89	118.2
Min.-Max.	16.0–317.8	19.0–1298.0	27.0–129.7	3.5–673.0
Averages for tuberculous guinea pigs with lesions in tissues listed	59.16	175.05	57.31	112.7
Min.-Max.	7.4–317.8	19.0–1298.0	27.0–129.7	3.5–673.0
Averages for tuberculous guinea pigs with lesions; not fed calcium	43.0	100.58	61.1	128.6
Min.-Max.	7.4–160.0	19.0–181.0	29.2–129.7	3.5–673.0
Averages for tuberculous guinea pigs with lesions; fed calcium	83.6	257.8	52.4	106.07
Min.-Max.	20.3–317.0	32.0–1298.0	27.0–91.3	22.1–278.0
Averages for tuberculous guinea pigs without lesions in tissues listed	25.0	All tuberculous guinea pigs have some enlarged lymph nodes	54.8	104.4
Min.-Max.	16.0–39.2		40.5–80.9	One guinea pig
Averages for tuberculous guinea pigs without lesions; fed calcium	24.9	All tuberculous guinea pigs have some enlarged lymph nodes	62.05	104.4
Min.-Max.	16.0–39.2		43.2–80.9	One guinea pig
Averages for tuberculous guinea pigs without lesions; not fed calcium	25.1	All tuberculous guinea pigs have some enlarged lymph nodes	40.5	Lesions in all guinea pigs not fed calcium
Min.-Max.	24.6–25.6		One guinea pig	

cium per 100 gm. and no. 49 with but 3.5 mgm. Much of this difference depends on the degree of tuberculous involvement, as shown in table 10, this seeming to have a far greater influence on the calcium content of the tissues than the administration of calcium.

Hence these experiments do *not* indicate that administration of large amounts of calcium causes larger amounts to be deposited in tuberculous lesions than will be the case in an animal on a normal diet which contains ordinarily adequate amounts of calcium.

TUBERCULOSIS OF THE TESTICLE

As it was not possible to separate cleanly tuberculous and nontuberculous portions of the same organ from one another in guinea pigs with systemic disease, in order to compare more accurately the influence of the tuberculous focus itself upon the calcium content of the tissue, attempts were made to secure tuberculosis of one of a pair of organs and to compare its calcium content with that of the normal mate. Doctor DeWitt first inoculated a series of guinea pigs directly into one lung, but it was not found possible in this way to secure a lung with extensive tuberculosis while its mate was normal or nearly so. Much greater success was obtained when the inoculation was made directly into one testicle. Although guinea pigs with systemic tuberculosis do not often exhibit extensive lesions in the testicle, this organ was found to be very susceptible to direct inoculation. Occasionally, a few small lesions were found in the uninoculated testicle, but these were not sufficient to affect the value of the experiment. This experiment was conducted as follows:

Fourteen animals were inoculated April 5, 1922. Each received 0.1 mgm., culture no. 4015, in the left testis. Seven were fed calcium daily from the day of inoculation. In all animals (except one) the inoculated testicle showed more evidence of tuberculous infection than its control. The exception was no. 82. Two small tuberculous nodules were visible in the noninoculated testis of no. 80. Two animals, nos. 79A and 79B, were much larger animals and were given 8 mgm. of the same culture. The animals were killed at the end of periods varying from 15 to 63 days, but the amount of involvement was by no means proportional to the duration of the infection. The results are shown in table 11.

Examination of this table reveals that in all cases the tuberculous testicle was larger than its mate, and with few exceptions contained

more calcium per unit of weight. This illustrates again the fact that tuberculous lesions take on calcium even when there is neither gross nor microscopic evidence of calcification, a property which seems to be common to necrotic tissues of whatever sort. This same difference is well shown in the two spleens that exhibited lesions which could be separated from the relatively normal tissue (table 7, nos. 37 and 42). Here the tuberculous portion contained very much more calcium than the nontuberculous portions of the same organ.

It so happens that the proportion of calcium averages somewhat higher in the testicles of guinea pigs not fed calcium than in those that did receive calcium. This, however, has little significance in so short a series, since in each group there is one specimen that has many times more

TABLE 11

GUINEA PIGS NOT FED CALCIUM						GUINEA PIGS FED CALCIUM					
Number	Length of experiment in days	Tuberculous testicle		Nontuberculous testicle		Number	Length of experiment in days	Tuberculous testicle		Nontuberculous testicle	
		Weight of dried testicle	Calcium mgm. in 100 gm.	Weight of dried testicle	Calcium mgm. in 100 gm.			Weight of dried testicle	Calcium mgm. in 100 gm.	Weight of dried testicle	Calcium mgm. in 100 gm.
92	45	0.2983	96	0.1985	67	79A	15	1.1853	1064	0.5268	37
89	46	0.3295	43	0.0543	47	79B	23	0.4523	41	0.2854	18
91	46	0.5176	1225	0.0897	504	85	31	0.2255	209	0.0640	120
93	47	0.2533	238	0.0672	20	84	45	0.2009	104	0.1345	126
90	49	0.1705	79.1	0.0693	47	82	49	0.4147	142	0.2154	64
88	51	0.2127	95	0.0536	52	81	49	0.1817	143	0.0747	100
87	55	0.1714	392	0.0523	52	83	53	0.1351	265	0.0527	100
						86	53	0.1991	209	0.1136	120
						80	63	0.3400	40	0.0695	120
Average		0.2647	309	0.0835	112			0.3483	246	0.1707	89

calcium than any of the others. If these are eliminated, the averages are not very different from one another. It will be noticed that in a few instances the nontuberculous testicle has the greater proportion of calcium; perhaps this depends on the existence in the tuberculous testicle of an early cellular stage of lesion without any necrotic tissue to increase the calcium content. However, we did not find the amount of calcium to vary directly with the amount of caseation visible macroscopically.

It has been found (46) that as little as 2 mgm. of calcium in an entire rabbit kidney may be detected microscopically as fine, dust-like particles, and 5 mgm. constitute a noteworthy microscopic calcification. This would be a proportion of about 100 mgm. of calcium per 100 gm. dry weight.

THE INFLUENCE OF CALCIUM ON THE COURSE OF EXPERIMENTAL TUBERCULOSIS

In some of the earlier experiments the impression was obtained that the tuberculous animals that received calcium did somewhat better than the controls, but whatever differences were observed were not constant or sufficiently marked to be convincing. In order to see whether any influence of calcium administration could be discerned in tuberculous guinea pigs, the following experiment was performed:

Twenty-four guinea pigs were inoculated subcutaneously with 0.005 mgm. of culture no. 4105. Twelve of these were fed calcium from the day of inoculation, while twelve were not fed calcium. Eighteen of these animals, evenly divided between calcium-fed and controls, died within 135 days. The lymph nodes, spleens, lungs, and livers of the

TABLE 12

Analyses of organs of calcium-fed and non-calcium-fed tuberculous animals

	CALCIUM IN 100 GMS. DRIED SUBSTANCE	
	Organs of guinea pigs *not* fed calcium	Organs of guinea pigs fed calcium
	mgm.	*mgm.*
Lungs (9)	206	107
Lymph nodes (9)	339	715
Spleens (9)	313	128
Livers (8)	59	62

treated and untreated animals were united and analyzed in two separate lots for calcium, so that each figure represents actually the average of eight or nine organs, without consideration of the degree of tuberculous involvement (table 12).

The results agree well with those obtained previously (tables 5 to 10), in showing that the highest calcium figures are given by the lymph nodes, which likewise have the most extensive tuberculosis. The spleen, with the next largest amount of tuberculosis, comes next, closely followed by the lung, while the liver shows relatively little calcium, in correspondence with its relative lack of tuberculous lesions. Here again the lymph nodes of the animals fed calcium show the highest calcium content, but as the liver and spleen exhibit the opposite relation it is probable that this excess in the lymph nodes comes from inhaled dust.

The average length of life of the calcium-fed guinea pigs was 92.5 days, while that of those not fed calcium was 74.4 days. Such a slight

difference is of no significance whatever, for nothing is less constant than the length of life of tuberculous guinea pigs even under identical conditions of dosage and environment.

In two of the animals which were not fed calcium the only evidence of tuberculous infection was found in an enlarged lymph node in the left groin, the site of inoculation. The lymph nodes, lungs and spleen of the 7 remaining guinea pigs showed marked progress of the infection. In the calcium-fed series one animal died of an unknown cause. The other 8 animals showed a severe generalized tuberculosis.

The livers in both series of animals were involved in 8 cases. If there was any difference in the degree of involvement of the organs, it was more marked in the animals that had been fed calcium.

A study of their weight curves (chart 1)[11] shows no essential difference in the two sets of animals. The observations of DeWitt (47) on the weight curves in tuberculous guinea pigs have indicated that these may be of some value in estimating the influence of treatment on the disease.

A study was made of the microscopic features of the lesions in the animals of this series, with particular reference to the degree of fibroplastic reaction to the tuberculous infection. Although this is to some extent determined by the length of time the animals lived, it was found that, when the sections were classified according to the amount of fibrosis in and about the lesions, somewhat more fibrotic lesions were found in the animals not fed calcium. Also, a few more foci showing microscopically visible calcification were found among the lesions from the animals that were not fed calcium, although the average life in this series was somewhat shorter. At least it can be said with certainty that in this experiment the administration of calcium has not reduced the amount of spread of the lesion nor has it led to a greater amount of fibroplastic tissue reaction.

In addition to the eighteen guinea pigs of this series described above there were six others that lived more than eight months. It so happened that three of these were animals that were being fed calcium and three which had no calcium. Here again the gross and histological appearances in the two groups of three animals each, showed no noteworthy differences except that as before there was, if anything, more fibrosis in the three animals that had not been fed calcium. Only one animal

[11] The numbers on the curves in this and succeeding charts indicate the number of animals furnishing the figures at this date.

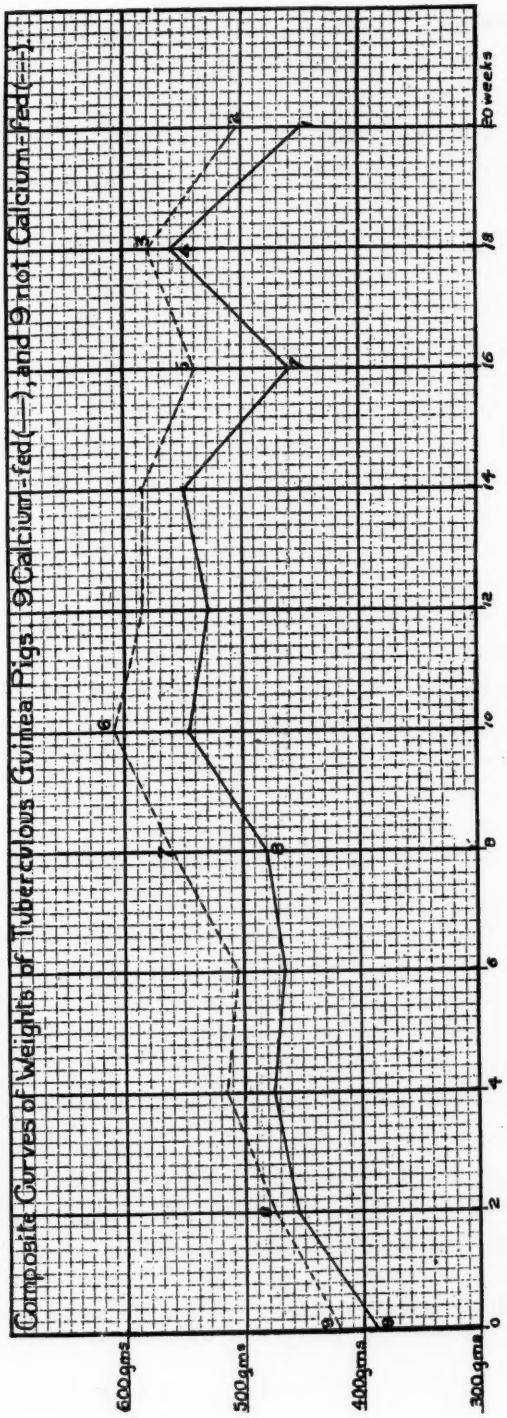
Composite Curves of Weights of Tuberculous Guinea Pigs. ⊙Calcium-fed(—), and ⊙not Calcium-fed(---).
20 weeks
18
16
14
12
10
8
6
4
2
0
600 gms
500 gms
400 gms
300 gms

CHART 1

showed calcific deposits visible under the microscope and this animal (no. 61) was one that had not been given calcium; there were large calcified areas in the spleen and a few small calcified areas in the lymph glands and lungs. The organs of the three animals of each series were analyzed together and the results are given in table 13. Corresponding to the microscopic findings, we have distinctly more deposition of calcium in the tissues of the animals that did not receive calcium.

TABLE 13

The calcium content of the organs of guinea pigs that lived longer than eight months after inoculation

	CALCIUM IN 100 GMS. DRIED TISSUE			
	Lungs	Lymph nodes	Spleens	Livers
	mgm.	*mgm.*	*mgm.*	*mgm.*
Calcium fed tuberculous, 62, 67, 71.....	174.4	450.2	233	75.0
Tuberculous, no calcium treatment, 56, 57, 61 ..	193.8	510.9	388	74.1

BLOOD CHANGES UNDER CALCIUM FEEDING

Having under observation a series of animals with and without tuberculosis, some of which were receiving calcium and some not, a study of the blood changes was considered worth making, especially because in this laboratory Dr. DeWitt and Miss Lauretta Bender have been carrying on an extensive study of the blood in tuberculous guinea pigs, a preliminary report of which was given at the 1922 meeting of the National Tuberculosis Association.

Chart 2 shows the coagulation time of the blood of these animals, and reveals that, contrary to expectation, the animals fed calcium showed a slower coagulation of the blood than the normal guinea pigs. While the tuberculous animals without calcium showed a much slower coagulation time than the normals, the calcium-fed guinea pigs with tuberculosis had a more rapid coagulation time than the tuberculous ones without calcium. The differences were, however, scarcely great enough to be of much significance. It may be recalled that in human tuberculosis the coagulation time has generally been found to be somewhat more rapid than normal (48).

Systematic blood counts were made and the results are shown in chart 3. It will be seen that the blood count was little altered as the tuberculosis progressed in these guinea pigs, and that there was no noteworthy

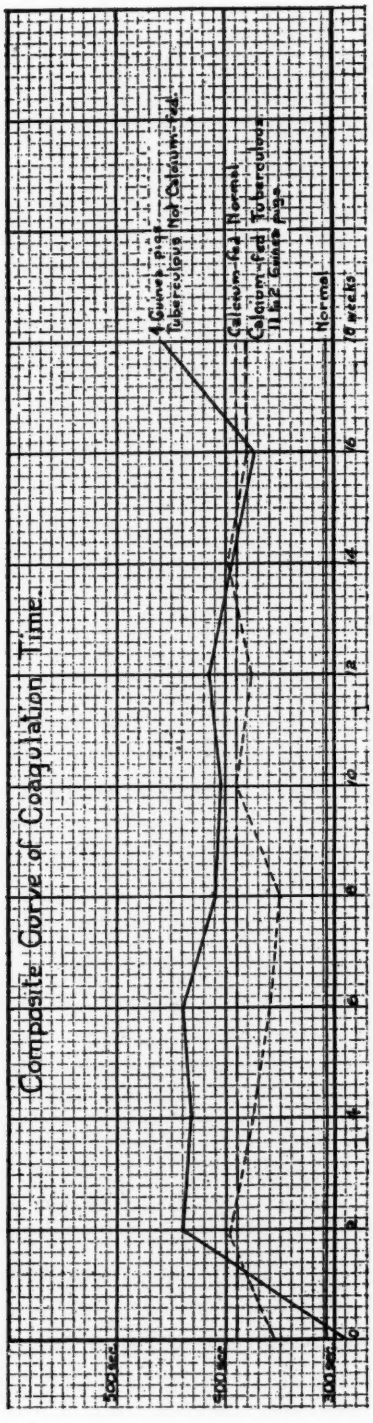
Composite Curve of Coagulation Time.
4 Guinea pigs Tuberculous Not Calcium-fed.
Calcium-fed Normal.
Calcium-Fed Tuberculous 11 to 12 Guinea pigs.
Normal
16 weeks
300 sec.
500 sec.
300 sec.

CHART 2

difference between the calcium-fed guinea pigs and those that did not receive calcium. Only the hemoglobin was altered. It ran steadily between 58 and 64 per cent in the animals fed calcium, but in those not fed calcium it started at 74 per cent and fell steadily to about 60 per cent, after which it continued at about the same level as in the calcium-

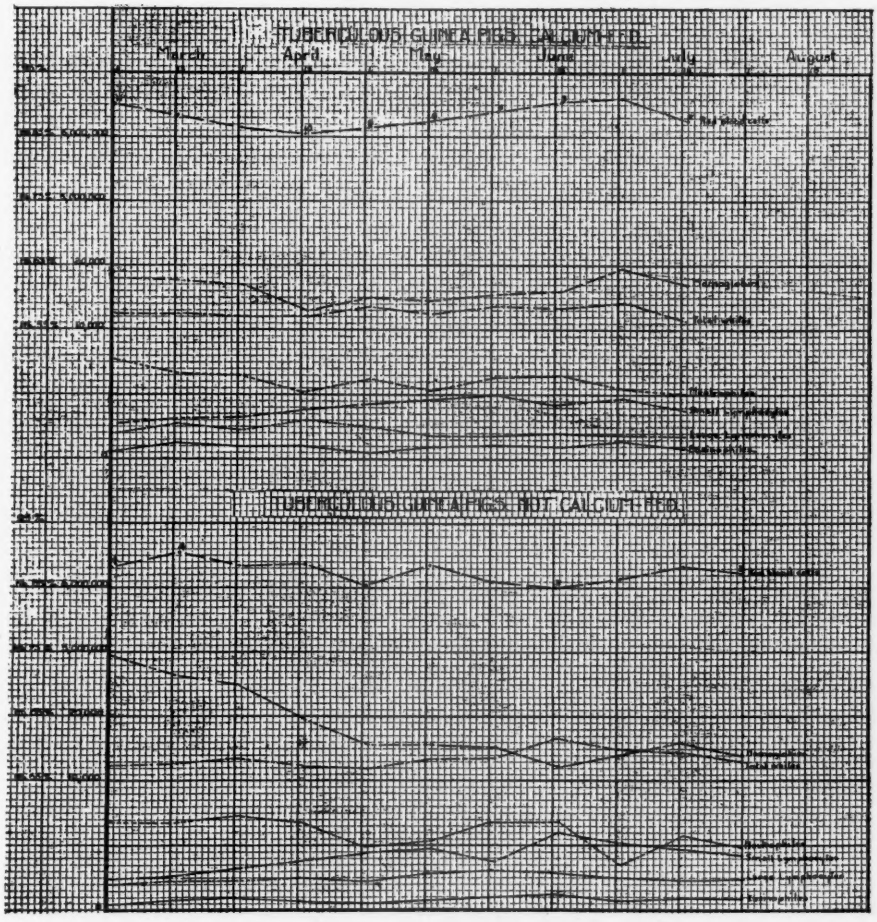

CHART 3

fed animals. This variation in the hemoglobin was not accompanied by any corresponding change in the number of red cells, which remained nearly constant. Usually the hemoglobin curve in guinea pigs has been found to start at a higher level, that is, from 80 to 90 per cent, and to fall in the acutely tuberculous guinea pigs somewhat more than the red cell count, as it did in these animals.

SUMMARY

A review of the literature on the therapeutic use of calcium in tuberculosis, and especially the experimental evidence on this subject, reveals the presence of a considerable body of clinical observation indicating a favorable effect of calcium therapy upon the tuberculosis itself as well as on certain of its complications, a certain amount of evidence that such favorable results are not obtainable, and a complete lack of convincing experimental evidence in support of calcium therapy. The hypothesis of a decalcification or demineralization in tuberculosis does not receive support from the most carefully conducted investigations. Also, the best chemical evidence is that there is no demonstrable deficiency of calcium in the blood or tissues in tuberculosis, and that feeding of calcium salts does not appreciably increase the amount of calcium in the blood when this is already normal.

Our own experiments have shown that guinea pigs on an ordinary laboratory diet exhibit some variations in the calcium content of the same tissue in different animals, and of different tissues in the same animals, yet, averaged together in a series of animals, the results are not far from the same, being generally lowest in the liver and highest in the lymph nodes, but not far from the same content as the blood, that is, 10 to 13 mgm. of calcium per 100 gm. moist weight.

Addition of calcium lactate to the diet of normal guinea pigs does not increase the amount of calcium to be recovered from most of the tissues. The kidneys may contain a little more, presumably because they are excreting any excess absorbed, and the lymph nodes usually show an increase, presumably from inhalation of dust.

Analysis of the entire bodies of mice (skin and intestines excluded) showed no more calcium in those that had been fed calcium lactate than in those on the standard diet.

Analysis of the tissues of guinea pigs with experimental tuberculosis shows a marked tendency to the accumulation of calcium in the tuberculous tissues, even when calcification is not visible microscopically. The amount of calcium deposition does not always vary in proportion to the extent of the tuberculous change in an organ. It was also found that the amount of calcium in the tuberculous lesions is not appreciably modified by feeding calcium lactate in addition to the usual diet. Sometimes the tuberculous tissues showed more calcium in the animals that received calcium and sometimes there was more in the control animals.

The tuberculous lungs show much less tendency to accumulate calcium than tuberculous spleens, livers or lymph nodes.

Direct inoculation of the testicle in the guinea pig was found to produce a marked involvement of the testicle, furnishing material that could be compared with the normal organ of the same animal. Here again there was a marked deposition of calcium in the tuberculous tissues, but not greater in the animals fed calcium than in the controls.

No demonstrable protective or curative effects could be observed in a limited number of tuberculous guinea pigs fed calcium as compared with the course of the disease in the control animals. There was no evidence that fibroplastic protective or healing reactions were increased by the feeding of calcium.

The blood cytology was not different in a series of calcium-fed tuberculous guinea pigs than in the control animals. The coagulation time in the calcium-fed guinea pigs was somewhat longer than in controls, but it was still longer in the tuberculous guinea pigs that were not fed calcium.

These studies, therefore, have failed to produce any experimental evidence which supports the empirical clinical evidence that calcium administration has a favorable influence upon the course of tuberculous infection. They do support the evidence of chemical studies that administration of calcium salts by mouth does not appreciably increase the amount of calcium in the blood and tissues, for this is normally maintained at a level not far below the saturation point of the body fluids.

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STUDIES ON NUTRITION IN EXPERIMENTAL TUBERCULOSIS

I. THE EFFECT OF THE FAT-SOLUBLE A VITAMINE ON TUBERCULOSIS OF THE GUINEA PIG, WITH ESPECIAL REFERENCE TO THE VALUE OF CODLIVER OIL IN EXPERIMENTAL TUBERCULOSIS[1]

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Codliver oil has long been regarded by clinicians to exert beneficial effects in chronic wasting diseases such as tuberculosis and rickets. It had been generally believed that, owing to the ease with which this substance is absorbed and assimilated, its nutritional value sufficiently accounted for its beneficial effects. In more recent times metabolism experiments in rickets and allied conditions, under the administration of codliver oil, have shown this substance to so alter the metabolism of inorganic salts as to effect a positive calcium balance (1) (2). The extensive researches of McCollum and his collaborators on the etiology and prevention of rickets have brought forth another aspect of the problem of codliver oil in this nutritional disorder which appears to have some bearing on the disease of tuberculosis. Studies on rickets by these investigators have indicated that, apart from its nutritional value, codliver oil possesses, in addition to its growth-promoting principle, certain other food accessories which appear to have a regulatory influence over the mineral metabolism of the body, having to do especially with calcium deposition (3). The tubercle is normally frequently the seat of calcium deposition, and the process of calcification of the tubercle is regarded as a means of spontaneous arrest of the disease. The possibility thus arises that codliver oil may alter the calcium metabolism in the tuberculous organism, as it has indeed been shown to do in the rachitic organism, and cause a deposition of lime salts in the tuberculous foci and thus effect a partial cure. If this hypothesis is correct, the beneficial

[1] Approved for publication by the Surgeon-General.

effects of codliver oil should be susceptible of demonstration by comparing the lesions of tuberculous animals treated with it with those of tuberculous animals wholly or partly deprived of the food accessories believed to be present in this oil.

Our knowledge of the value of codliver oil in tuberculosis is largely based on clinical impressions. Very little carefully controlled experimental work on this subject appears to be available. Weigert (4) conducted feeding experiments on pigs and concluded that the tuberculous process generalized more rapidly in his animals fed on a strictly carbohydrate diet than in those receiving a liberal amount of fat. He did not however use codliver oil as a source of fat. Wells (5) made a comparative study of codliver oil and a certain brand of an emulsion of codliver oil on the growth of the normal and tuberculous pig, and claims to have obtained better results with the emulsion than with the oil. The experiments were not carefully controlled and cannot be regarded as decisive.

Concerning the rôle of vitamines in infection and immunity, it should be noted that Biondo (6) found that when pigeons were deprived of the water-soluble B factor they lost their natural immunity against anthrax. While these experiments were in progress, Gloyne and Page (7) published a paper on the effect of the fat-soluble A factor on tuberculosis of the white rat, an animal most suitable for feeding experiments; but their results were inconclusive, since they failed to produce an infection either in the nontreated or in the treated animals.

Another consideration which led the writer to investigate this problem is the reported effects of codliver oil and its derivatives on the tubercle bacillus and on tuberculous infection. Williams and Forsyth (8) found that tubercle bacilli grown in the presence of codliver oil lost their acid-fast properties, presumably because of the disintegration of their waxy components, this probably making them more vulnerable. Further, a derivative of codliver oil, sodium morrhuate, has been used clinically in tuberculosis, and some authors claim to have obtained favorable results from it (9) (10) (11) (12) (13).

In this work an attempt has been made to determine the effects of codliver oil administered orally, without, as well as with, the simultaneous administration of calcium, on tuberculosis of the experimental animal. The oil was administered in one series of experiments to tuberculous guinea pigs kept on a normal diet, and in another series to animals which were deprived wholly or partly of the fat-soluble A food accessory.

In each case the groups of animals receiving codliver oil were controlled by similar groups receiving olive oil, a substance which, as far as is known, is free from the fat-soluble A factor.

1. THE EFFECTS OF CODLIVER OIL ON TUBERCULOSIS OF THE GUINEA PIG KEPT ON A NORMAL DIET

In this series of experiments three groups of 26 guinea pigs each, and two groups of 10 each were used. The animals of these groups were of approximately the same age and weight, and were kept in lots of 3 or 4 in a cage, under uniform conditions, on a diet of oats and cabbage with hay bedding. The weights of all the animals were recorded weekly. Group *A* of this series consisted of 8 males and 2 females which served as normal controls. A like number of animals were used in group *B*, which received in addition to their regular diet a daily dose of 0.5 cc. of codliver oil emulsion administered orally with a medicine dropper. The emulsion was prepared according to the *U.S.P.* formula, with the exception that the syrup was omitted. From these two groups of experiments it was planned to determine the effect of codliver oil on the normal animal kept on a normal diet.

The animals of group *B*, receiving codliver oil for a period of nearly fourteen weeks, appeared in very good condition throughout the experiment, and seemed to do perhaps a trifle better than the controls of group *A*, as will appear from curves *A* and *B* of chart 1, which represent the average weight curves of the two groups of experimental animals. Groups *C*, *D* and *E* consisted of 26 animals each, all of which were inoculated intraperitoneally with 0.5 mgm. tubercle bacilli, human strain H 37. Group *C* served as controls. The animals of group *D* received a daily dose of 0.5 cc. codliver oil emulsion *per os*, and those of group *E* received weekly intravenous injections of 60 mgm. per kilo of calcium lactate in addition to the codliver oil feeding as of group *D*. The treatment was begun immediately after inoculation and was continued to the end of the experiment. Curves *C*, *D* and *E* of chart 1 show the average losses in weight of the animals of the corresponding groups. In order to obtain strictly comparable results, only young growing animals of approximately the same age and weight, and showing at autopsy approximately the same degree of tuberculous involvement, were selected.

Table 1 shows in detail the number of animals, their sex, and extent of tuberculosis found at autopsy of the animals whose weight charts

are represented by curves *C*, *D* and *E*. It will be noted that the treated animals, especially those receiving codliver oil and calcium lactate, were at a distinct disadvantage as compared with the controls. The same

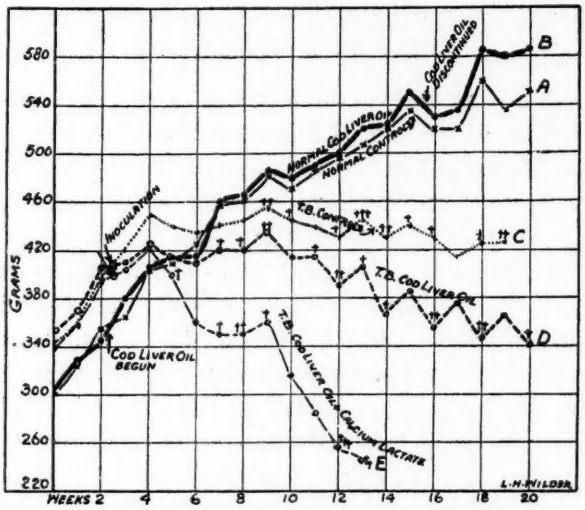

CHART 1. EFFECT OF CODLIVER OIL ON NORMAL AND ON TUBERCULOUS GUINEA PIGS KEPT ON A LIBERAL DIET OF OATS, CABBAGE AND HAY

A: Average weight curve of ten normal nontreated guinea pigs.

B: Average weight curve of ten normal guinea pigs treated with codliver oil.

C, *D* and *E:* Average weight curves and mortality rate of controls, codliver oil, and codliver oil with calcium treated animals, infected with tuberculosis.

†: Indicates death of an animal.

TABLE 1

Number, sex and extent of tuberculosis of the guinea pigs represented in curves C, D and E of chart 1

GROUP	NUMBER OF ANIMALS	SEX	EXTENT OF TUBERCULOUS INVOLVEMENT AT AUTOPSY		
			Local	Moderate generalization	Extensive generalization
C	14	All males	1	2	11
D	14	All males	1	4	9
E	10	6 males, 4 females	0	3	7

feature appears upon examination of the mortality curve of these three groups of animals as shown in chart 2. The death-rate curves of the controls and of those treated with codliver oil (*C* and *D* respectively) are nearly alike, while curve *E*, representing the mortality rate of the guinea

pigs treated with codliver oil and calcium lactate, is somewhat steeper and much shorter. The average length of life for the controls was 97 days as against 83 days for the group treated with codliver oil, and 61 days for those treated with codliver oil and calcium lactate (table 2).

It is not probable that the primary toxicity of the calcium lactate shortened the life of the animals of the last group, although it may have lowered their resistance and thus rendered them more susceptible to the disease, as is indeed generally true of most remedies thus far used in the

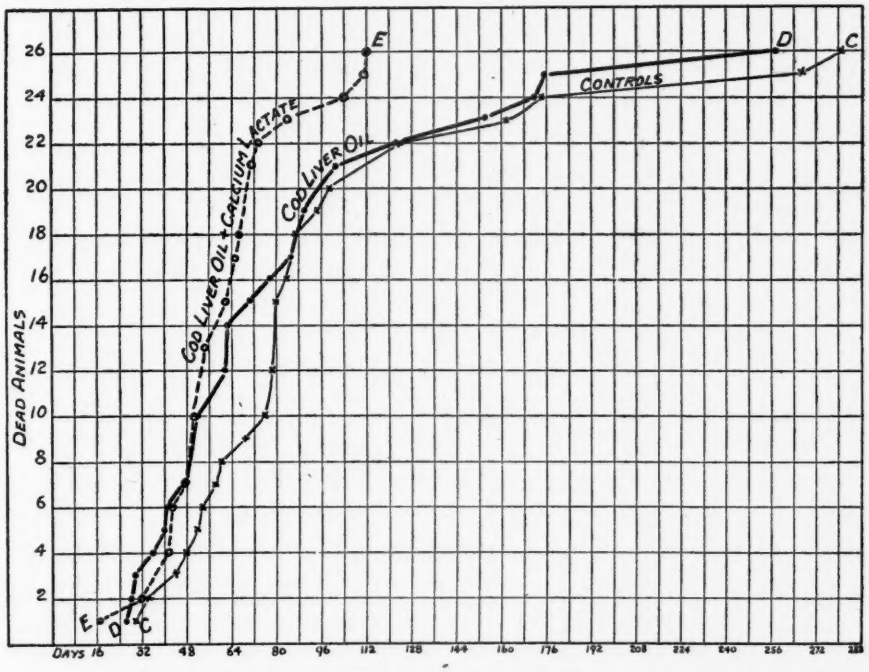

CHART 2. MORTALITY RATE OF CONTROLS, CODLIVER OIL, AND CODLIVER OIL WITH CALCIUM TREATED GUINEA PIGS, INFECTED WITH *Bacillus Tuberculosis*, HUMAN TYPE, AND KEPT ON A LIBERAL DIET OF OATS, CABBAGE AND HAY

treatment of experimental tuberculosis in the guinea pig. This appears to be the correct explanation, for, in spite of the relatively short life of the animals of this group, a larger percentage of them showed at autopsy extensive tuberculous involvement than the animals of the other two groups (see table 3).

All the animals of this series were carefully autopsied after death, and the extent of tuberculous involvement of the various organs noted. In most instances histological sections were prepared for microscopic

TABLE 2

Duration of life of guinea pigs of groups C, D, and E

C CONTROLS		*D* TREATED WITH CODLIVER OIL		*E* TREATED WITH CODLIVER OIL AND CALCIUM LACTATE	
Number	Days	Number	Days	Number	Days
1	30	1	27	1	17
2	34	2	28	2	32
3	45	3	30	3	35
4	48	4	36	4	41
5	52	5	40	5	42
6	54	6	41	6	42
7	57	7	48	7	48
8	60	8	49	8	49
9	69	9	52	9	51
10	76	10	52	10	51
11	79	11	62	11	54
12	79	12	62	12	54
13	80	13	63	13	55
14	80	14	63	14	62
15	80	15	71	15	62
16	84	16	78	16	63
17	85	17	85	17	65
18	87	18	90	18	67
19	95	19	90	19	71
20	99	20	101	20	71
21	123	21	101	21	71
22	123	22	123	22	74
23	162	23	154	23	84
24	175	24	172	24	104
25	275	25	175	25	111
26	289	26	265	26	112
Average.....	97		83		61

TABLE 3

Extent of tuberculous involvement in groups C, D and E

PERCENTAGE OF ANIMALS HAVING:	GROUPS		
	C Controls	*D* Treated with codliver oil	*E* Treated with codliver oil and calcium lactate
	per cent	*per cent*	*per cent*
Local tuberculosis.....	4	8	4
Moderate generalization.....	27	46	19
Extensive generalization.....	69	46	77

study, to determine especially whether there was any evidence of healing or calcification. This was never observed in any of the aforementioned groups. Table 3 is arranged to show the relative amount of tuberculosis of the several groups. It will be noted that 77 per cent of the animals treated with codliver oil and calcium lactate presented extensive generalization of the disease, as against 69 per cent of the controls, and as against only 46 per cent of the group treated with codliver oil. The disease apparently did not spread as rapidly in the codliver oil animals as it did in the nontreated controls, although the death rate of these two groups was approximately the same.

It may be concluded from this series of experiments that the administration of codliver oil does not lengthen the life of the tuberculous guinea pig kept on a normal diet, although it appears to have some slight influence on the disease process. The intravenous administration of calcium lactate along with the feeding of codliver oil shortens the life of the animal and appears to hasten the tuberculous process, perhaps by lowering the resistance of the experimental animal.

2. THE EFFECT OF CODLIVER OIL ON TUBERCULOSIS OF THE GUINEA PIG MAINTAINED ON A DIET LACKING IN THE FAT-SOLUBLE A FACTOR

In this series of experiments it was planned to investigate the influence of a diet deficient in the fat-soluble A food accessory on the progress of the disease, and, further, to determine the effect of codliver oil administered to tuberculous animals on such a diet. It seemed probable that by depriving the animal of this food accessory the disease would run a more rapid course, and it could thus be possible to demonstrate the benefits that might accrue from the administration of codliver oil. The problem seemed further worthy of consideration, for it seemed probable that a diet might be worked out for the guinea pig that would hasten the progress of the disease and which could be utilized where the inoculation is made for diagnostic purposes.

The selection of a satisfactory diet deficient in the fat-soluble A food accessory for the guinea pig is a very difficult matter. Most of the experimental nutrition work dealing with this vitamine has been carried out upon the rat, which thus far has not proved very suitable for tuberculosis experiments. The feeding habits of the guinea pig are, as is well known, quite different from those of the rat, and diets designed to control the A vitamine in the rat are generally quite unsuitable for the guinea pig.

After considerable preliminary experimentation, it was found that, by using rolled oats and wheaten bran as the basal diet, it was possible to supplement this in various ways, so as to produce a more or less adequate diet, wholly or partly deficient in the fat-soluble A factor. These diets, while not complete, nor such as to sustain the life of the animal indefinitely, were sufficiently adequate, however, to keep them alive long enough to permit of some definite conclusions to be drawn.

The fat-soluble A factor was either entirely omitted from the diet of the controls or included only in small amounts, while to the treated animals it was supplied in abundance in the form of codliver oil emulsion (0.5 cc. daily).

The most striking manifestation of the deficiency of the fat-soluble A factor in the diet of the non-tuberculous controls was the effect of it on the weight curves. The animals promptly began to fall off in weight as soon as they were placed on the diet, due for the most part to reduced food consumption. After a few days they resumed eating, but the weight curve remained practically stationary. Eye lesions were not observed. Beading of the costochondral junctions and fragility of the bones were frequently observed in the animals on the vitamine A free diet. This generally became manifest in animals that came to autopsy at least six to eight weeks after they had been put on the diet. Beading of the costochondral junctions was not observed among the animals on similar diets supplemented with codliver oil. The beading of the costochondral junction was evidently the result of the faulty diet, and is suggestive perhaps of a rachitic condition induced in the animals, owing to the lack of the antirachitic factor. Tozer (14) states that deformities at the costochondral junctions in the guinea pig, caused by lack of fat-soluble A, are often indistinguishable from scurvy. It is doubtful whether the condition observed here is scurvy, for the animals received sufficient amounts of orange juice; they showed no other symptoms suggestive of scurvy; and, furthermore, no such changes occurred in the costochondral junctions of animals receiving codliver oil.

The administration of codliver oil along with the deficient diets did not restore the normal weight curves, although it distinctly prolonged life. This raises the question of whether the oil was utilized by the animals. The answer must be in the affirmative, since it protected the animals from developing deformities at the costochondral junctions. It would seem that the growth-promoting factor in codliver oil does not affect the guinea pig in a manner as it does other animals, such as the rat.

Further, it does not appear to affect the growth of the guinea pig in a manner comparable with the effects of the fat-soluble A factor present in milk and green vegetables, as shown by the experiments of Hume (15).

This series of experiments consisted of four groups, with four lots of animals in each group. The animals were kept in individual cages under uniform conditions of ventilation, light, etc. Sawdust bedding was used throughout. All the animals of this series had been bred on the premises, their weight curves were carefully watched from the time of birth, and only those showing a satisfactory rate of growth were used. Young growing males, usually of from 250 to 350 gm., were selected. The animals were weighed once a week. The four lots of animals of each group were arranged as follows: *Lot 1:* 4 normal controls on the particular basal diet supplemented with codliver oil; *lot 2:* 4 normal controls on the same basal diet, plus olive oil emulsion; *lot 3:* 8 guinea pigs inoculated with tuberculosis and fed on the basal diet supplemented with codliver oil; and *lot 4:* 8 animals inoculated with tuberculosis and fed on the basal diet plus olive oil. All the animals of lots *3* and *4* of the four groups were inoculated at the same time with the same dose of the same strain of tubercle bacilli (0.5 mgm. H 37, human, intraperitoneally). One additional lot of guinea pigs was inoculated under identical conditions, but kept on a normal diet of oats, 40 gm. of cabbage per day and hay bedding.

The animals were carefully autopsied, and the extent of tuberculous involvement was noted and recorded in percentages according to an arbitrary standard adopted, which was as follows: The principal sites of tuberculous involvement in the guinea pig, namely, the spleen, liver and peritoneum were given four counts each; the omentum and pancreas, and the lungs three counts each; and, lastly, two counts for the lymph nodes, thus making a total of twenty counts of 5 per cent each. To illustrate: A liver that presented a few yellowish small spots was given one count of 5 per cent. One presenting numerous miliary tubercles was given a count of 10 per cent. If it presented a few small caseating areas in addition to the above it was counted as 15 per cent. Lastly, a liver presenting extensive caseous necrosis was given the maximum number of counts, that is, four, or 20 per cent. A similar arbitrary scale was adopted for the organs and tissues enumerated above. The extent of involvement expressed in percentages was plotted on the subjoined charts, and it is believed to give a fairly true representation of the facts.

The basal diet used in this series of experiments consisted of rolled

oats and wheaten bran. As McCollum, Simmonds and Pitz (16) pointed out that the proteins of the oat kernel are inadequate, an attempt was made to supplement it with gelatin, but this was found unsatisfac-

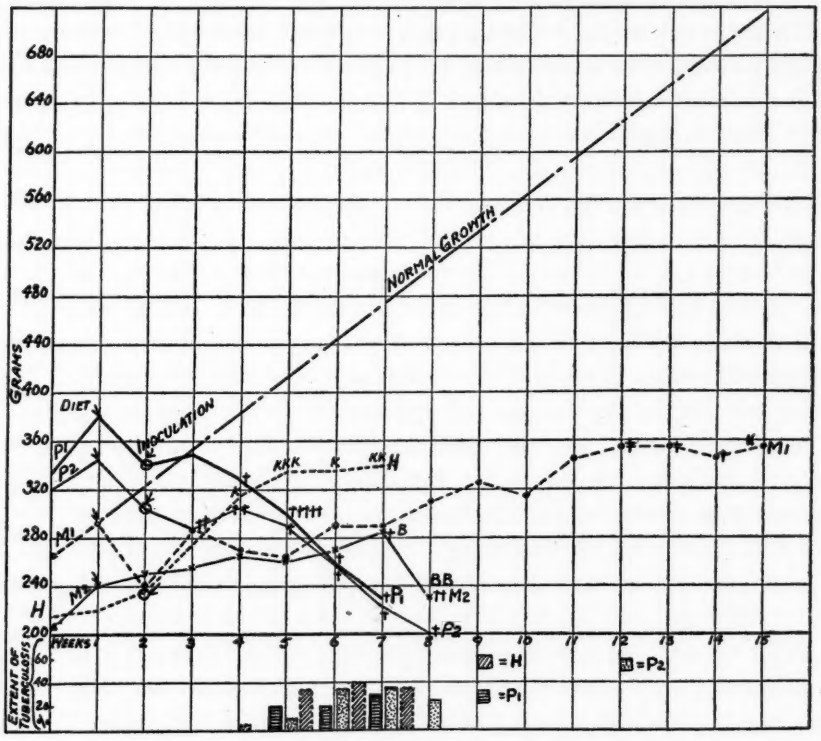

CHART 3. EFFECT OF CODLIVER OIL ON NORMAL AND TUBERCULOUS GUINEA PIGS FED ON A VITAMINE *A* DEFICIENT DIET

(For details of diets see text)

M1 and *M2:* Average weight curves of nontuberculous guinea pigs on the deficient diet, treated with codliver oil and olive oil, respectively.

P1 and *P2:* Average weight curves of tuberculous guinea pigs on the deficient diet, treated with codliver oil and olive oil, respectively.

H: Average weight curve of nontreated tuberculous guinea pigs fed on a liberal diet of oats, cabbage and hay.

In this, and in the subsequent charts the vertical columns indicate in percentages the extent of tuberculous involvement of the animals of the corresponding groups having come to autopsy at the particular period.

†: Indicates death of an animal.

K: Indicates that an animal was killed.

B: Indicates beading of the costochondral junction.

Normal Growth curve indicates the average gains in weight of normal guinea pigs fed on a liberal diet of oats, cabbage and hay.

tory. Better results were obtained by supplementing the diet with casein. The diet of the animals of group *1* (chart 3) thus consisted of rolled oats 80 per cent, wheaten bran 10 per cent, casein 10 per cent, water *ad libitum*, 5 cc. orange juice daily, and 1 cc. of a salt mixture containing 100 mgm. of calcium lactate and 10 mgm. sodium chloride. These amounts of calcium and sodium represent approximately the difference between their content in 10 to 20 gm. rolled oats and 40 gm. cabbage per day, which gives a practically normal growth curve, and in the present diet. Oats and bran contain an abundance of the other minerals, such as Mg, K, P, and S (Sherman and Gettler (17)), and it did not seem necessary to complicate the salt mixture further. The emulsion of codliver oil, in amounts of 0.5 cc. daily, was administered to one set of animals, and an emulsion of olive oil was given in like amounts to the other set.

The diet of group *2*, the results of which are shown in chart 4, consisted of rolled oats, wheaten bran and water *ad libitum*, 2 gm. of skimmed milk powder per day, representing approximately 20 cc. fat-free milk, and 5 cc. orange juice daily. Codliver oil and olive oil were administered as in the last group. The mineral content of the diet appeared to be sufficient, theoretically at least.

The diets of groups *3* and *4* were designed to allow a minimal amount of the fat-soluble A factor, in the hope that this would permit of a longer period of observation. In group *3*, 20 cc. of whole milk were allowed daily, in addition to the rolled oats and wheaten bran which were administered *ad libitum*. This contained about one-third the amount of fat-soluble A necessary to promote normal growth (Hume (15)). Similarly, in group *4*, 15 gm. of the green leaves of raw cabbage were allowed daily in addition to the basal diet. No mineral salts were administered with this diet. Orange juice was administered to the animals of group *3*, since the amount of milk given is not sufficient to protect the guinea pig from scurvy. The amount of cabbage allowed the animals of group *4* was regarded as ample (18) to protect them from scurvy, and the orange juice was omitted. Paper pulp was allowed for roughage to animals of groups *1*, *2* and *3*.

The results of these experiments are summarized in the accompanying charts. Curves *M 1* and *M 2* on chart 3 represent the average weight curves of nontuberculous animals of group *1*, on a deficient diet treated with codliver oil and olive oil, respectively. For comparison, the curve of normal growth representing the average weight curve of 8 normal

guinea pigs on a diet of oats, 40 gm. cabbage per day and hay bedding is given. It will be noted that the animals on codliver oil fared better than those on olive oil. Beading of the costochondral junctions was observed in all of the latter and in none of the former lot. Curves *P 1* and *P 2* represent the average weight curves and mortality rate of codliver oil and olive oil-treated tuberculous animals, respectively. The two curves run practically parallel. For comparison curve *H* is

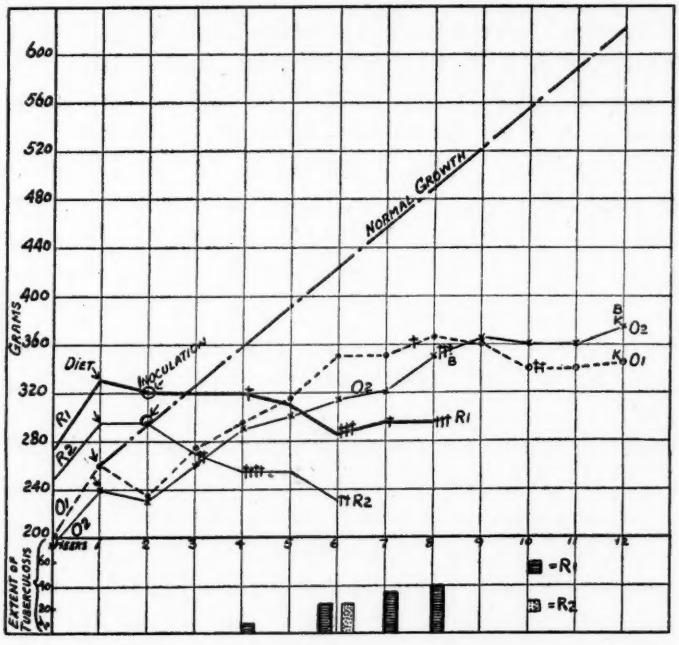

CHART 4: EFFECT OF CODLIVER OIL ON NORMAL AND TUBERCULOUS GUINEA PIGS FED ON A VITAMINE *A* DEFICIENT DIET

O1 and *O2:* Average weight curves of nontuberculous guinea pigs treated with codliver oil and olive oil, respectively.

R1 and *R2:* Average weight curves of guinea pigs infected with tuberculosis and treated with codliver oil and olive oil, respectively.

inserted, which represents the average weight curve of 7 guinea pigs, infected with tuberculosis under the identical conditions as those of *P 1* and *P 2* but kept on a liberal diet of oats, 40 gm. of cabbage per day and hay bedding. Whenever an animal of lots *P 1* and *P 2* died, the poorest of lot *H* was killed and autopsied. The extent of tuberculous involvement of these three groups is thus shown graphically in percentages. There appears to be very little difference in the amount of

tuberculous involvement, though there is a very pronounced difference in the weight curves and mortality rate of *P 1* and *P 2* on the one hand and that of *H* on the other.

Chart 4 likewise summarizes the results of the administration of codliver oil to another group of animals on a deficient diet. Curves *O 1* and *O 2* are weight curves of nontuberculous guinea pigs of group *2*,

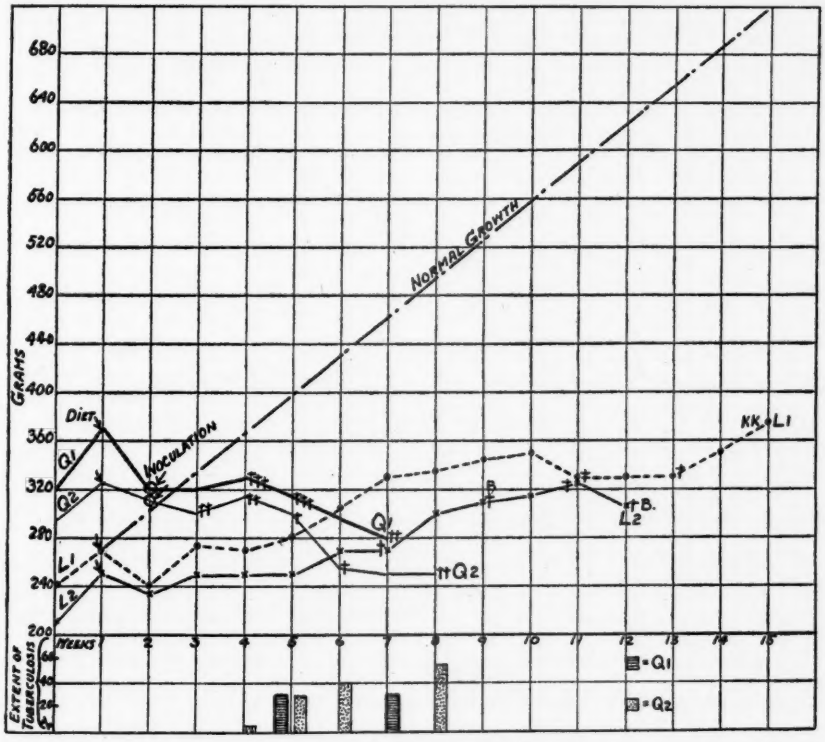

CHART 5. EFFECT OF CODLIVER OIL ON NORMAL AND TUBERCULOUS GUINEA PIGS FED ON A DIET PARTLY DEFICIENT IN VITAMINE *A*

L1 and *L2:* Average weight curves of nontuberculous.

Q1 and *Q2:* Average weight curves of tuberculous guinea pigs treated with codliver oil and olive oil, respectively.

on codliver oil and olive oil, respectively, and *R 1* and *R 2* are weight curves of tuberculous guinea pigs under similar treatment. Two of the animals of lot *O 2* showed beading of costochondral junctions, while none of the *O 1* lot showed deformities. The tuberculous guinea pigs of lot *R 1* appear to have fared a little better than those of *R 2*. The difference is not sufficiently marked, however, to be decisive.

Charts 5 and 6 show the results of codliver oil administration to normal and tuberculous guinea pigs on diets partly deficient in the fat-soluble A factor. Two of the animals of lot *L 2*, group *3*, treated with olive oil, showed deformities of the costochondral junctions; none were observed in lot *L 1*, treated with codliver oil. Curves *Q 1* and *Q 2* (chart 5) representing the weight gains and losses of tuberculous guinea pigs, on the deficient

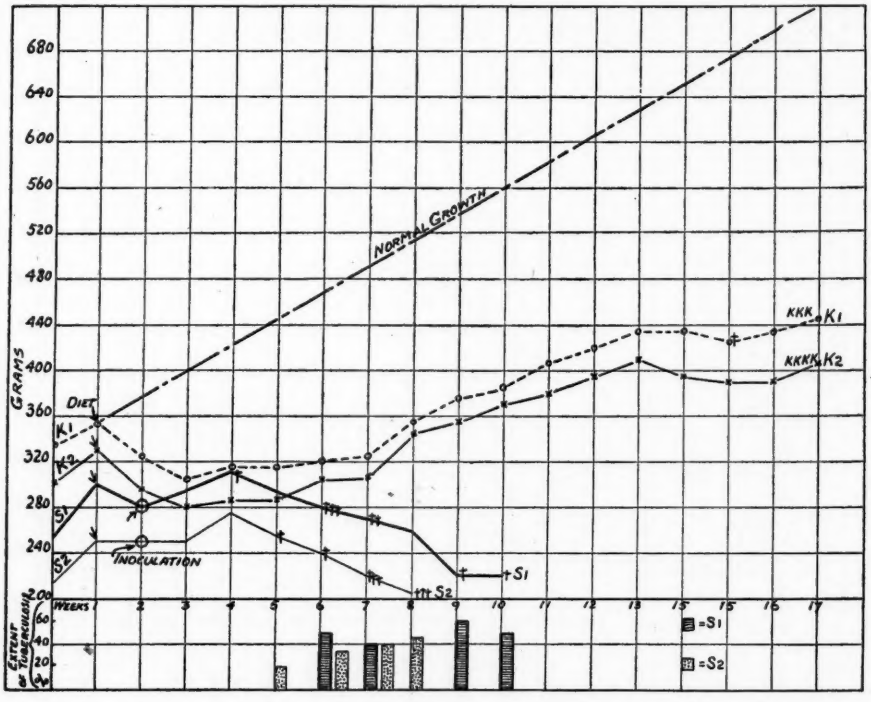

CHART 6. EFFECT OF CODLIVER OIL ON NORMAL AND TUBERCULOUS GUINEA PIGS FED ON A PARTLY VITAMINE *A* DEFICIENT DIET

K1 and *K2* represent average weight curves of nontuberculous, and *S1* and *S2* of tuberculous guinea pigs treated with codliver oil and olive oil, respectively.

diet and treated with codliver oil and olive oil, respectively, run nearly parallel. No great difference in the amount of tuberculosis was noted in these two lots. Nearly all the animals of lots *K 1* and *K 2* (group *4*) survived the period of observation of sixteen weeks and were chloroformed. None showed beading at the costochondral junctions. The weight curves of these two lots of animals are nearly parallel. These animals showed a slight but definite gain in weight. The weight curves

of the tuberculous animals on this diet, supplemented with codliver oil and olive oil, respectively, (*S 1* and *S 2*), also run nearly parallel; and here, as in the last groups, the extent and progress of the disease were approximately alike in the codliver oil and olive oil-treated animals.

SUMMARY

The results of these experiments are negative in so far as concerns the action of codliver oil on tuberculosis of the guinea pig. This substance has not shown itself definitely beneficial, either as regards weight curve, length of life or extent of the disease process, in experimentally infected guinea pigs kept on a normal or a deficient diet. There has been no evidence of deposition of calcium in tubercle of the guinea pig when this element was administered along with the codliver oil. Codliver oil appears to have, however, a definite though slight effect on the nutrition of the nontuberculous guinea pig, especially when the latter is maintained on a diet deficient in the fat-soluble A factor. The life of such animals when fed codliver oil is distinctly prolonged, and, as has been pointed out earlier, deformities do not develop at the costochondral junctions as they do in the nontreated animals. Why guinea pigs infected with the tubercle bacillus do not react to codliver oil as regards their length of life, at least, is difficult to see, unless that the infection is so overwhelming that an agent having a slight beneficial effect in the nontuberculous animal becomes useless in the tuberculous organism. It is not unlikely, however, that the remedy under consideration is only partly utilized by the guinea pig. It is quite probable that the white rat with its more or less natural resistance to the tubercle bacillus and greater ability of utilizing fats might yield more decisive results. Experiments along this line are in progress in this laboratory.

I wish to express my indebtedness to Messrs. Earl G. Hendrick and F. Spindle for their untiring efforts in carrying out the feeding experiments.

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EXPERIMENTAL TUBERCULOSIS IN RATS ON VARIED DIETS

PROTEIN AND SALT FACTORS

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In the absence of any adequate therapy, either specific or medicinal, in human tuberculosis, general measures calculated to increase the natural powers of resistance constitute an accepted mode of treatment. Rest, whether for the whole body, or more particularly for the diseased lung, as provided by artificial pneumothorax; an abundance of fresh air in well-ventilated rooms or out of doors, modified according to temperature in selected cases; and a diet looking to the building up of the body depleted by disease, are the measures generally employed. Regarding the last there is no complete agreement. Overfeeding or merely adequate feeding, whether in three meals or at more frequent intervals, has been tried. The constitution of the diet has also been the subject of debate. Overfeeding with milk and eggs, high total protein or especially a high meat protein; disregard of carbohydrates or advocacy of low carbohydrates; disregard of fats, or advocacy of a high fat percentage, have all had their supporters. While these regimens are concerned chiefly with the treatment of tuberculous patients, they have also been advised as prophylactic measures looking to the prevention of clinical disease in persons, more especially children, considered to be "below par" and usually already infected with the tubercle bacillus.

Where the factors are as many and as uncontrolled as they are in chronic conditions such as human tuberculosis, accurate deductions are necessarily difficult or impossible, as evidenced by the absence of unanimity regarding modes of treatment among tuberculosis experts. It has seemed, therefore, that an attempt might profitably be made to test the reaction to infection with the tubercle bacillus in animals under controlled conditions of environment and diet. A coöperative investigation was arranged with the Department of Chemical Hygiene, since the experience of the members of that department assured the prep-

aration of experimental animals by methods which would make possible the assignment of any defect in their vitality to specific chemical faults in their diets.

Guinea pigs were discussed as subjects, but because of the difficulties of dietetic work with this species it was decided to attempt the experiments with rats. This was done with some hesitation, in view of the prevailing idea of the refractoriness of rodents to tuberculosis and skepticism as to the possibility of setting up the disease in these animals. A preliminary series of rats was infected subcutaneously in the groin with heavy and light doses, respectively, of three strains of tubercle bacilli obtained from the Saranac Laboratory through the kindness of Doctor Baldwin. They were the bovine strain, *B 1*, and two human strains, *H 37*, virulent for guinea pigs and *R 1* of very low virulence for guinea pigs. Gross and microscopic examinations made at weekly intervals for four weeks pointed to the higher virulence for rats of the *B 1* strain and this microörganism was therefore used in the following experiments.

For the preparation of the animals, a diet was selected which could readily be modified with respect to its content and quality of protein, without changing in a significant degree the value of any dietary factor. In a single instance sodium chloride and calcium carbonate were omitted (low salts diet).

The basal diet consisted of rolled oats, gelatin, casein, sodium chloride, calcium carbonate, dextrin and butter fat. It contained an abundance of *fat-soluble A* and *water-soluble B*, but was essentially lacking in *water-soluble C*, the antiscorbutic substance. This is, however, a matter of no importance in studies with the rat, since this species is capable of synthesizing this vitamine and is not susceptible to scurvy.

The animals were about six months old when they were restricted to the experimental diets. The element of growth was therefore not included, and any difference in the vitality of any of the experimental groups depended upon the extent to which their respective diets were suitable for the maintenance of health in the adult. The sexes were separated so that the records were not complicated by reproduction, and the animals were restricted to the experimental diets for seven to eleven weeks before the inoculation.

For each diet a larger group was infected subcutaneously in the right groin with a bacillary emulsion, in amounts proportional to the weight of the rat, while a smaller group was left uninfected and served as diet

controls. The weights were recorded every two weeks. At intervals of one week for five weeks, starting from the day of infection, a lot consisting of a stated number of infected animals and one uninfected animal on the same diet was killed with ether and carefully autopsied. A sixth lot was sacrificed after five months, a few of the animals necessitating examination before that time. The gross lesions were noted and blocks of tissue fixed in formol-Zenker's fluid for histological study. Sections were stained with haematoxylin and eosin and, in addition, those of the lymph nodes, lungs and spleen were stained for bacteria by the Ziehl-Neelsen method for acid-fast bacilli and by the MacCallum method for Gram-positive and Gram-negative organisms. With this material it was possible to study any progressive tissue changes due, first, to the presence of the tubercle bacillus and, secondly, to the diet alone in animals on a given diet, and, finally, to compare tissue reactions to the infection and to the diet, respectively, in animals on different diets.

The reaction was, in a general way, the same in the different groups of rats and resembled, up to a certain point, tuberculous lesions in other animals. Soon after subcutaneous inoculation into the groin, tuberculous tissue appears in the connective tissue of the groin, and in the superficial inguinal and iliac lymph nodes of the infected side. Later, nodules may appear in the tracheal lymph nodes. Probable tubercles were present in the spleen in one case after five weeks and in another at four and a half months. Everywhere the nodules consist of typical epithelioid cells with vesicular oval nuclei, which in some cases later become more slender and densely stained. They are situated as a rule in the cortex of the lymph nodes and eccentrically in the follicles, more rarely in the medullary portion of the glands. In the earlier lesions the epithelioid proliferation is not well circumscribed (figure 1). Later, the nodules in the lymph nodes become clearly demarcated (figures 2, 3, and 5), and still later the sharp margins are sometimes broken by lymphocytic invasion. Tubercle bacilli of characteristic morphology are easily found in the tubercles in most cases by the Ziehl-Neelsen (figure 8) and MacCallum (figure 9) stains. By the latter method the granules are stained a purplish black and the body of the bacillus sometimes a clear red. In rare instances the bacilli appear as solidly staining, slender, Gram-positive rods. The usual form was the short bacillus characteristic of the bovine strain, but occasionally long slender forms appeared with as many as eight or ten beads in one bacillus. It is common to

find more bacilli by the Gram stain than by the acid-fast stain, where they are demonstrated in the same tissue by both stains. No bacteria other than tubercle bacilli were found in any case in this series. Giant cell formation and necrosis occurred but rarely. Connective tissue encapsulation was never observed. Instead, the tubercles undergo gradual disintegration and shrinkage. The first changes in the fresh tubercle are a paling or decrease in number of the epithelioid nuclei and perhaps an inwandering of a few lymphocytic cells. The nodule assumes a yellow tinge, faint at first, but gradually becoming more marked, until there remains only a very small body of a dark, dirty yellow color, containing no nuclei or at most one or two, and these often round rather than oval. Parallel with this tissue disintegration the bacilli become fewer in number; then only acid-fast fragments or detritus or a few Gram-positive granules can be found; and finally no material of probable bacillary origin appears. Differences in this progression of events as shown between rats on different diets may now be considered.

Four groups in all were studied: (1) a group on a normal maintenance diet with an adequate percentage of protein, (2) a high protein group, (3) a low protein group, and (4) a group on low salts. Fats, carbohydrates and the so called vitamines have not been tried out, except in so far as the low protein group may be considered to be on a high carbohydrate diet.

The three protein groups were infected and autopsied together. The diets, begun seventy-two days before infection, are given in the following table:

Protein diets

	ADEQUATE	HIGH	LOW
	per cent	*per cent*	*per cent*
Rolled oats	40	40	40
Casein	5	10	
Gelatin	5	10	
NaCl	1	1	1
$CaCO_3$	1.5	1.5	1.5
Dextrin	44.5	34.5	54.5
Butter fat	3	3	3

An emulsion of the *B1* (bovine) strain was prepared by grinding culture material in a glass mortar until a fine suspension was obtained. This

was centrifugalized, to throw out the unavoidable clumps, and a measured volume of the supernatant fluid was spread on a slide (previously rubbed with Mayer's albumen) and the bacilli stained and counted. Estimates were made of the few remaining small clumps. On the basis of this count a saline suspension of known bacterial content was prepared. One and a half million bacilli per 100 gm. body weight was the infecting dose, inoculated subcutaneously into the right groin. For each protein diet 18 rats were infected and 6 served as controls. They were autopsied in six lots, each lot consisting of three infected animals and one uninfected control.

After one week tuberculous tissue was found in the right inguinal subcutis, and in the right inguinal and iliac lymph nodes. The rats killed at two and three weeks showed no infiltration around the inguinal nodes, pointing to a clearing up of the reaction in this location. Contradictory evidence was found in one adequate protein rat at four weeks, two high protein rats (one at five weeks and one at five months), and in a low protein rat at three months, all of which still showed tuberculous tissue in the subcutis. The epithelioid cell reaction in the lymph nodes after one week tended to be somewhat diffuse and ill-defined (figure 1); but after two weeks more sharply demarcated tubercles were found (figure 2), resembling the tuberculous tissue in the hyperplastic type of human tuberculous lymph nodes (figure 3). In the rats, however, the lymph node tubercles were fewer in number and less in relative extent and were situated, in the far greater number of instances, only in the follicles and but rarely in the lymph cords or sinuses. After three weeks, degeneration of the tubercles began, as evidenced by slight yellowing which became more definite at four weeks as a more marked pigmentation, and in a paling or decrease in number of the epithelioid nuclei. In a number of instances the tubercles in the iliac nodes were in a fresher state than those of the inguinals, pointing to a later involvement of the iliacs. In only one instance did the left iliac node show tubercles. Tubercles were observed in the tracheal lymph nodes for the first time at five weeks (figure 5). They were similar in structure but less in number than those of the other glands. The sixth and last lots of the protein series were kept for five months, except one high protein rat killed at two months, and three low protein rats, one of which was killed at three months and two at four and a half months. In these sixth lots small, deeply pigmented nodules were found in the same location as the undoubted tubercles from the earlier autopsies and they most probably

represented degenerated tubercles. They were easily distinguished from the darker pigment-bearing cells common in the spleen and often present in the lymph nodes. Inasmuch, however, as no material was at hand in which transition stages between the earlier and the last pictures could be traced, and no undoubted bacilli could be demonstrated in these late-stage pigmented areas, a definite decision as to their nature was impossible.

No constant variation in tissue reaction appeared as between the rats in the three protein groups. Such differences as occurred seemed an expression of the individual reaction independent of the dietetic factor. Thus, where one rat presented a diffuse ill-defined epithelioid infiltration, another showed sharp, clear-cut epithelioid tubercles, and the character of the reaction was the same in all the tissue of any one animal. The weight curves, too, were entirely lacking in any evidence pointing to a distinction between infected and uninfected rats on the same diet, or between corresponding infected or uninfected rats on different diets. Kidney lesions were found in scattered cases but did not fall in line with the factors of infection or of diet. The liver, however, did so, in that the high protein livers were entirely normal; the adequate protein livers, especially in the last three lots, showed moderate fatty degeneration, while those of the low protein group showed a more advanced fatty change. Only two low protein rats escaped this condition entirely. One infected low protein rat, killed at four and a half months and described as "very old," presented an atrophy of the parenchyma in both kidney and liver. In the kidney, groups of convoluted tubules were represented by scanty, pale, amorphous remains of protoplasm peppered with shrunken pycnotic nuclei. In the liver the groups of degenerated cells consisted each of a cell wall, a well-preserved nucleus located centrally or a little to one side, while the cytoplasm was reduced to a scanty, irregular rim of granular material about the nucleus, extending outward in irregular radiating strands, to become continuous with a little of the same granular material clinging to the inner surface of the cell wall. The vacuolation of the protoplasm was not sharp as in fatty degeneration. In neither the kidney nor liver was there collapse of the tissues.

When the fourth diet (low salts) was to be studied, so few rats were available that no normal diet controls could be run and only twelve rats could be infected, reducing the six autopsy lots each to two infected and one uninfected animal. To approximate the conditions obtaining in the adequate protein rats of the earlier series as closely as possible, the

bacillary emulsion was prepared from the same strain in exactly the same way and the same dose of one and a half million bacilli per 100 gm. body weight into the right groin was inoculated seven weeks after the diet had been given to the whole group. The low salts diet resembled the adequate protein diet as shown in the table:

	ADEQUATE PROTEIN	LOW SALTS
	per cent	*per cent*
Rolled oats	40	40
Casein	5	5
Gelatin	5	5
NaCl	1	
$CaCO_3$	1.5	
Dextrin	44.5	47
Butter fat	3	3

One week after infection a diffuse and extensive tuberculous infiltration of the inguinal subcutaneous tissue developed. In one of the rats the marginal sinus of an inguinal lymph node was crowded with epithelioid cells, among which were found large numbers of undoubted Gram-positive tubercle bacilli. The lymphoid substance of both the inguinal and iliac nodes was quite normal. Killed after two weeks, both the infected animals showed the subcutaneous infiltration in the groin; one, epithelioid cells in the marginal sinus of the inguinal node and the other, tubercles in the substance of the iliac node containing Gram-positive tubercle bacilli. The fact that the inguinal node appearing in the section in the latter case was clear is of no significance, since it is common to find one inguinal node riddled with tubercles while a second node from the same animal and situated immediately next to it may show no lesion whatever. After three weeks neither rat showed widespread subcutaneous infiltration, but in one there was extensive infiltration immediately about the node and extending into the hilum and through the marginal sinus. There were a few small areas of epithelioid cell necrosis. In the other rat the extensive tuberculous tissue about the node contained a few giant cells, which while small were of the characteristic Langhans type, whereas the node itself was clear. After four weeks one rat still had an extensive involvement of the subcutaneous tissue, an extensive diffuse infiltration of the inguinal node and a similar picture in the iliac node. At the fifth week there was a large number of tubercles in the inguinal nodes but none in the iliacs. In both rats there were early signs

of disintegration of the inguinal node tubercles. Of the sixth lot, one rat killed at three months presented a few dark yellow tubercle-like lesions in both inguinals and iliacs, while the tubercles of the second rat killed at fourteen weeks were still in an earlier stage of better preservation. In no rat of the low salts series were tubercles found in the tracheal nodes. Neither kidneys nor livers showed any significant changes.

Comparing the three protein series with the low salts series, it is evident that in the latter the tissue reaction to tuberculous infection is more extensive and more diffuse, the disseminated lesions appear later and the elimination of tuberculous tissue is slower. It seems unlikely that all of these differences are referable to a variation in the virulence of the infecting microörganism, and the difference is so general in the whole group that it can more reasonably be ascribed to the other common factor, that of diet. In the absence of a simultaneous control series on normal diet a positive assertion can, however, not be made.

In no case were undoubted tubercles found in the lungs. Tubercle-like nodules were present in several instances, but as they were not quite characteristic and bacilli could not be found, it was thought more likely that they represented part of another and distinct pathological condition present in the lungs of more than half of the rats. It is possible that animals kept for a longer period might have shown the pulmonary lesions reported by Galli Valerio.

In all of the rats it is evident that we deal with a highly resistant host. As far as they go the tissue reactions to the bacillus and the mode of dissemination resemble corresponding phenomena in other more susceptible animals. Compared with those of the guinea pig, the lesions are slight in extent. The injury does not advance as a rule, or extensively, to caseation or even giant cell formation. The bacilli appear to be overcome directly by the host and are not merely walled off by a connective tissue capsule. The prevalent idea of the resistance of the rat is, however, an exaggerated one and may be due in part to the fact that many lymph nodes showing no gross enlargement or caseation may still be riddled by small tubercles. Failure to make microscopic examinations in such cases would leave the examiner with the impression of normal tissues. In this connection it is interesting to recall the early doubts as to the propriety of the use of the highly susceptible guinea pig for experimental tuberculosis intended to throw light on the problems of human infection.

SUMMARY

Four series of rats were infected with bovine tubercle bacilli by subcutaneous inoculation into the groin and the progress of the infection was studied at intervals of from one week to five months after infection.

Three series were on diets of varying protein content, adequate, high and low. No significant difference was noted in the reaction to infection in the three groups, either in the general condition and weight curves or in the gross and microscopic autopsy findings.

The fourth series was on a diet deficient in salts but otherwise adequate. These animals showed a more diffuse and extensive local reaction at the site of inoculation, a slower dissemination and a slower elimination of the lesions.

In all four series there was a high resistance to tuberculous infection. As far as it went, the reaction was similar in character and progress to that found in less resistant animals, but the process never advanced in any of the sixty-six infected animals of this experiment to extensive necrosis. Instead, the invading bacilli appear to be overcome directly rather than to be merely walled off. No instance of connective tissue encapsulation was observed. Instead, there was slow shrinkage and absorption of the tuberculous tissue accompanied by disappearance of the tubercle bacilli.

Fig. 1

Fig. 2

Fig. 3

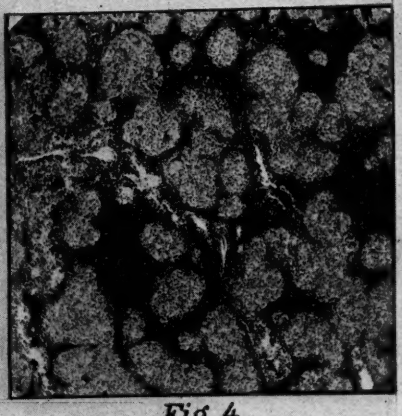

Fig. 4

Fig. 5

Fig. 6

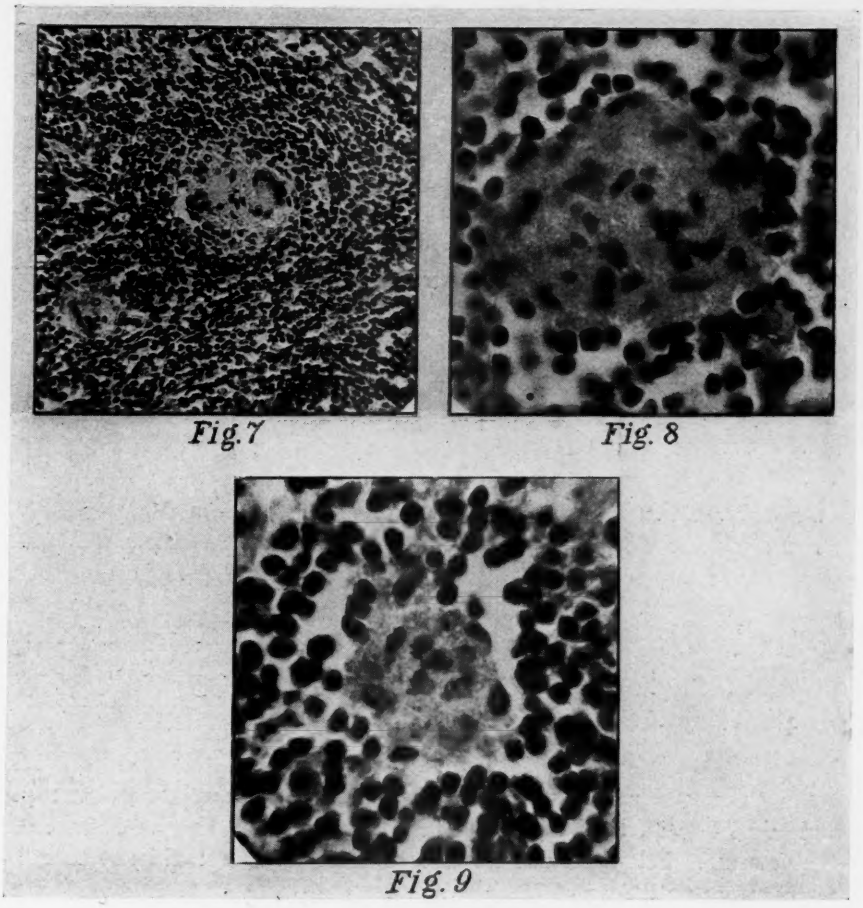

Fig. 7

Fig. 8

Fig. 9

FIG. 1. RIGHT INGUINAL LYMPH NODE. ONE WEEK AFTER INFECTION. LOW PROTEIN DIET. × 30

There is a diffuse, ill-defined infiltration with epithelioid cells

FIG. 2. RIGHT INGUINAL NODE. TWO WEEKS AFTER INFECTION. HIGH PROTEIN DIET. × 27

The epithelioid cells have become grouped in sharply demarcated tubercles

FIG. 3. TRACHEAL NODE. FIVE WEEKS AFTER INFECTION. HIGH PROTEIN DIET. × 40

Small clear-out epithelioid tubercles similar to those in other lymph glands

FIG. 4. TUBERCULOUS HUMAN LYMPH NODE, HYPERPLASTIC TYPE. × 30

The epithelioid tubercles are clear-out with a tendency to giant cell formation

FIG. 5. RIGHT INGUINAL NODE. THREE WEEKS AFTER INFECTION. LOW SALTS DIET. × 30

Diffuse widespread epithelioid infiltration within and about node

FIG. 6. DETAIL OF FIGURE 5, SHOWING CHARACTER OF EPITHELIOID CELLS OF THE PERIGLANDULAR TUBERCULOUS TISSUE. × 200

FIG. 7. DETAIL OF FIGURE 5, SHOWING EPITHELIOID TUBERCLES IN SUBSTANCE OF LYMPH NODE WITH GIANT CELL FORMATION. × 200

FIG. 8. HIGH POWER PICTURE OF LYMPH NODE TUBERCLE STAINED BY THE ZIEHL-NEELSEN METHOD, SHOWING ACID-FAST TUBERCLE BACILLI. × 800

FIG. 9. HIGH POWER PICTURE OF LYMPH NODE TUBERCLE STAINED BY THE MACCALLUM METHOD, SHOWING GRAM-POSITIVE TUBERCLE BACILLI. × 800

A CASE OF PRIMARY LOWER LOBE TUBERCULOSIS

WITH ONSET OF ACTIVITY IMMEDIATELY AFTER PROPHYLACTIC TYPHOID INOCULATION

I. S. KAHN

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Miss W. C., trained nurse, age thirty-one, was seen August 7, 1922. *Family history:* Positive for recent tuberculosis. *Past History:* Measles in childhood and influenza in 1918, but no other illnesses suggesting tuberculosis.

Present illness: Had had full opportunity for infection from nursing a sister with tuberculosis complicated by tuberculous pyopneumothorax, requiring many withdrawals of pus for over a year previous to her death in December, 1921. Returned then to her work. Was very intelligent and fully aware of her liability to infection, and on the lookout for its slightest evidence. Had no cough, stood her work well, and gained constantly in weight and strength. On July 29, 1922, took typhoid prophylactic vaccine, owing to contact with that disease. Felt miserable for two or three days, but did not take her temperature until August 2, when it was found to be 102°. Noticed cough for the first time on August 1. Cough has persisted since, rather tight and dry in character, with very scanty expectoration. She became alarmed at persistence of cough and reported on August 7 for examination.

General examination unimportant. *Chest examination:* Left side normal. Right showed normal physiological dulness and broncho-vesicular breathing at apex, but no râles. Posterior from base to lower angle of scapula and lower half of axilla, as far forward as anterior axillary line, there was dulness, and, even without cough, numerous fine crepitant and subcrepitant râles, more numerous following cough. There was no change in the character of the respiratory murmur or whispered voice. Diaphragm excursion was, of course, very limited.

X-ray stereo (Dr. W. S. Hamilton) showed moderate tuberculous infiltration extending from hilum to periphery of the lower lobe on right, with one small old cavity in the upper portion of infiltration. Upper lobes clear. Sputum scanty and mucoid, but revealed tubercle bacilli.

This case is reported owing to the known rarity of the type. (Rosenblatt (1) reports only 3 cases in 1000 consecutive admissions at the Bed-

ford Hills Sanatorium.) I have met with only one other case (2) in my last 450 cases of tuberculosis. This case also suggests the possibility of the lighting up of an old dormant process following typhoid prophylaxis, inasmuch as the cavity present, as revealed by the X-ray, is obviously old. No respiratory infection or unusual exertion, and apparently perfect health preceded the sudden tuberculous activity in this instance; in fact, this case is rather remarkable for the complete absence of all premonitory symptoms, a positive sputum being secured after only

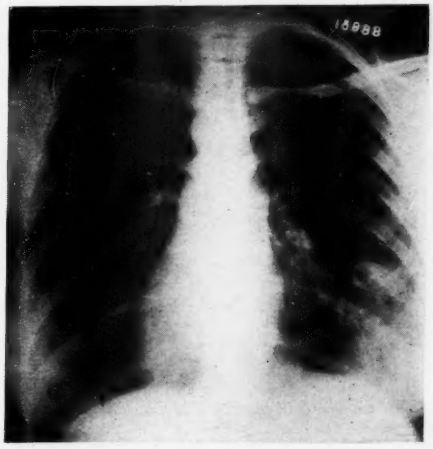

FIG. 1. TUBERCULOUS INFILTRATION WITH CAVITY FORMATION IN LOWER LOBE OF RIGHT LUNG

seven days of cough. For this reason the sudden high temperature immediately subsequent to the typhoid inoculation and its prompt subsidence to normal in five or six days suggest decidedly a nonspecific general and focal reaction, the absence of a previous chest examination alone rendering this debatable.

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A CASE OF CLOSED TUBERCULOUS PYOPNEUMOTHORAX

TREATED BY FORMALDEHYDE-GLYCERINE INJECTIONS WITH RECOVERY

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All workers in the field of pulmonary tuberculosis are agreed as to the ordinarily hopelessness of this condition, in spite of the gradual cessation of the purulent exudate in a small percentage of cases either under rest or following repeated removal and replacement by air or oxygen. The apparently complete recovery in this case through the use of formaldehyde-glycerine injections, after all other methods had failed, justifies, I feel, its being reported, corroborating the excellent results secured with this method of treatment by Kalb (1) in seven similar cases.

J. A. T., farmer, age thirty, first seen December 9, 1920. Previous duration of illness one and a half years. He gave a history of artificial pneumothorax treatment of the left side for six weeks, with seven air injections in all in a period of three weeks. A day or two after the last injection he developed sudden pain in the injected side, with high temperature and shortness of breath, and was decidedly sick (probably spontaneous pneumothorax). In the last few days had felt much better; temperature normal the last two days.

Examination revealed a moderately sick man, with distinct evidences of air and considerable fluid in the left pleural cavity. The heart was displaced far to the right. The right lung showed moderately numerous, typical, fine indeterminate râles, with dulness and bronchovesicular breathing above the third rib. The X-ray corroborated the physical findings that the pleural cavity was over half-full of fluid and pneumothorax compression was excellent.

On December 11 a few cubic centimetres of clear straw-colored fluid were withdrawn for diagnosis and 100 cc. of air introduced into the chest to an average pressure reading of +1. In order to maintain this intrathoracic pressure of +1, 25 to 100 cc. of air were introduced above the fluid level on December 9, 18 and 28, January 14, February 4 and 26, and March 27, the fluid itself not being touched, but remaining constantly at a high level, with no tendency to absorption in spite of strict bed rest, though the patient during

this time continued in excellent condition, free from fever, gaining in weight and almost free from cough and expectoration.

About the middle of April the patient began to have afternoon fever and was not feeling quite so well. On April 24 the chest was tapped and 250 cc. of cloudy fluid were withdrawn. This quickly reaccumulated, so that on May 2 tapping was again required and 1000 cc. of fluid were withdrawn and replaced by 400 cc. of air. This procedure had to be repeated twice at weekly intervals.

On May 16, 875 cc. of rather thin dirty pus were withdrawn, containing tubercle bacilli, and 650 cc. of air were introduced. It was then decided to replace pus with air regularly. Working along these lines, pus was withdrawn and air introduced to 0 intrathoracic pressure as follows: May 23, 320 cc. of pus withdrawn, May 30, 130 cc., June 6, 35 cc., June 14, 150 cc., June 24, 700 cc., June 30, 450 cc., and July 4, 150 cc. During this time the patient was slowly failing and evidently the pus formation was showing little tendency to diminish. It was then determined, in addition to removing pus and replacing it by air, to introduce into the pleural cavity, following each operation, about 2 to 4 cc. of saturated alcoholic solution of methylene blue, according to the method of Rosenblatt and Stivelman (2). The details of this treatment follow:

July 9, 300 cc. pus withdrawn, air in to 0, 2 cc. methylene blue introduced.

July 16, 200 cc. pus withdrawn, air in to 0, 3 cc. methylene blue introduced.

July 22, 200 cc. pus withdrawn, air in to 0, 4 cc. methylene blue introduced.

August 11, 100 cc. pus withdrawn, air in to 0, 4 cc. methylene blue introduced.

August 30, 200 cc. pus withdrawn, air in to 0, 4 cc. methylene blue introduced.

September 14, 180 cc. pus withdrawn, air in to 0, 4 cc. methylene blue introduced.

October 13, 310 cc. pus withdrawn, air in to 0, 4 cc. methylene blue introduced.

November 2, 285 cc. pus withdrawn, air in to 0, 4 cc. methylene blue introduced.

Up to this point the patient had done well. He ran no fever, gained a little in weight and had almost no cough. Repeated fluoroscopic examinations between operations showed very little fluid in the pleural cavity, and intervals between fluid withdrawals were being gradually but appreciably increased. However, immediately after the treatment of November 2, his fever returned, and he was obviously losing, and apparently no longer deriving benefit from the methylene blue. A few more trials of it were given as follows:

November 6, 525 cc. very thick pus out, 4 cc. methylene blue introduced.
November 10, 500 cc. very thick pus out, 4 cc. methylene blue introduced.
November 19, 750 cc. very thick pus out, 4 cc. methylene blue introduced.
November 22, 620 cc. very thick pus out, 4 cc. methylene blue introduced.

Obviously, the purulent exudate was now forming far more rapidly. Owing to the known poor results of thoracotomy in these cases, it was decided, before resorting to this measure, to exhaust every other possibility. My attention was then called to the article of Kalb (3) on Smith's method of treatment of tuberculous empyema by the use of formaldehyde-glycerine injections, and this treatment was then adopted.

On November 24, 120 cc. of very thick putty-like pus was withdrawn and 40 cc. of 2 per cent formaldehyde-glycerine solution injected into the pleural cavity, along with 50 cc. of air to neutral pressure. Considerable distress, pain and shortness of breath developed within a few hours and temperature rose to 101° for two days, during which time the patient felt very miserable.

On November 28 at 10 A.M., 300 cc. of very thin pus were withdrawn and 125 cc. of 2 per cent formaldehyde-glycerine injected. Intense reaction followed within a few hours, the patient becoming desperately ill, with intense pain, dyspnoea and extremely rapid pulse. The temperature was around 102°. At 3 A.M. of November 29, 600 cc. of thin serosanguinous fluid, containing a few blood clots, were withdrawn with immediate relief, the temperature remaining up for only two or three days thereafter.

On December 11, on account of slight fever, the chest was again explored, and 500 cc. of dark serosanguinous fluid withdrawn, evidently containing altered blood. Introduction of formaldehyde-glycerine was not deemed necessary.

Where fluid removal had been required every three or four days, it was now noted that the accumulation was much slower, the temperature was normal and the patient was feeling much better. However, after about three weeks, slight fever was again noticed, with increase of fluid. On January 3, 1922, 625 cc. of very thin pus were removed and 100 cc. of 1 per cent formaldehyde-glucerine solution introduced. Within the next few hours, as before, pain, shortness of breath and fever came on, and the usual rapidly forming exudate was detected. Effort was now made to relieve the intrathoracic pressure by air withdrawal. A manometric reading of average +9 cm. was secured and 300 cc. of air were withdrawn to 0 pressure with marked relief. Four days later, on account of persisting slight discomfort, 625 cc. of very thin serosanguinous fluid were withdrawn, containing little or no evidence of pus. On account of chest soreness it was deemed best to omit the usual formaldehyde-glycerine injection.

Following this fluid withdrawal, the patient did excellently. There was no fever nor cough, weight gain began and the general condition became very

satisfactory. However, return of the exudate was noted at the end of three weeks, and on January 31, 350 cc. of very thin serosanguinous fluid were withdrawn, containing, macroscopically, no pus. Air as usual was introduced to 0 pressure. Formaldehyde-glycerine was deemed unnecessary and none has since been used.

Since then the patient has convalesced rapidly requiring only refills of air to maintain compression at ordinary intervals, at which times fluid has been searched for and removed as follows, the fluid invariably being clear straw colored:

February 22, 200 cc.	May 29, 50 cc.
March 29, 120 cc.	July 3, none.
April 24, none	August 10, none.

Fluoroscopic examination, August 10, showed the pleural cavity completely free from fluid. Clinically, the patient is doing very well. He has gained about twenty pounds since the inception of formaldehyde-glycerine treatment. He has no cough, is feeling well and strong and is doing some work. The condition of the right lung has remained unchanged. Cure of the tuberculous empyema is apparently complete, no pus having formed in the last eight months.

The pus, which at the inception of the formaldehyde-glycerine treatment was so thick and putty-like that it could only with extreme difficulty be extracted through a no. 13 gauge needle, rapidly became very thin, then serosanguinous, as described by Kalb, finally clear straw-colored and then disappeared completely.

The complete success in this case, after the demonstrated lamentable failure of all other approved methods, would indicate for this treatment its place in handling tuberculous empyema, and render this complication a little less hopeless in its outlook.

There is one precaution to be observed. Following each introduction of the formaldehyde-glycerine, a comparatively enormous exudate is thrown out with startling rapidity, and, inasmuch as it is necessary in these cases to maintain the compressed lung in that state, a negative pressure following the removal of fluid, before the formaldehyde-glycerine mixture is introduced, is risky. For this reason, through the development of this exudate, within a few hours a high positive intrathoracic pressure is induced and the reaction is terrific, the clinical symptoms imitating completely those of a sudden spontaneous pneumothorax. This should be anticipated and arrangements made to reëstablish com-

fort by the reduction of this pressure through air and, if necessary, fluid removal.

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THE NATURE OF TUBERCULOSIS OF THE EYE

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Many physicians, who deal with tuberculosis in general, are not cognizant of the fact that tuberculosis occurs in the eye as a clinical entity. It is important, therefore, that attention be called to this phase of the disease. It is not the purpose of this paper to deal with tuberculosis in all details that might be considered from the standpoint of the ophthalmologist, but rather to convey enough general information concerning eye tuberculosis to be of value to the specialist whose main activities lie in chest conditions of a tuberculous character. He, however, should incidentally be measurably conversant with all other manifestations of tuberculosis in the body; not that he may be called upon to treat these conditions, but rather that in recognizing them he will be able to obtain proper and qualified aid from the ophthalmologist, at the time of the earliest appearance of the eye condition and when it is still in a remedial stage. The data in this paper constitute a summary of a study of the literature and experience with clinical cases, besides conclusions drawn from experimental animal investigations of tuberculosis of the eye.

Tuberculosis of the eye occurs at all ages, but it is found most often in young adults. It occurs in both sexes about equally. In the eye it is either a primary or secondary condition. If primary, the eye is the first organ in the body to be involved. The secondary type is more common, although in a study of the literature of the past one is led to believe that this is not the case. The reason for this is that there is often difficulty in locating the primary focus in the secondary variety and frequently it is impossible. Primary tuberculosis of the eye is of exogenous origin and is found in the exposed parts: the eyelids, conjunctiva, cornea and lachrymal sac are the common sites. Primary intraocular tuberculosis, not associated with trauma, is rare.

Either inactive or latent foci of tuberculosis in some parts of the body are the primary seats from which infection of the eye occurs in secondary

tuberculosis. Clumps of tubercle bacilli which pass into the blood from the primary focus are carried to the eye, accidentally lodge in ocular blood vessels, undergo proliferation and produce eye tuberculosis. All parts of the organ, except the lens, may be attacked in this manner.

Ocular tuberculosis occurs in acute, subacute and chronic types. The acute type manifests itself as tubercles of the choroid, associated with an acute miliary tuberculosis or with tuberculous meningitis; or as an acute involvement of any of the ocular structures which often terminates in destruction of the eyeball and necessitates enucleation. It is the usual rule that the acute variety is associated with active tuberculosis elsewhere in the body. In chronic ocular tuberculosis the disease is often confined, at least in the beginning, to one portion of the eye. As a rule there is little or no pain, and often the involvement is quite extensive before the subject is aware of the presence of any trouble. The progress of the disease is often slow. Slight activity may be present over a period of months or even years, and recurrence of activity after apparent cures are not uncommon. One cannot be certain that as long as the patient lives the disease will not again become active.

LOCATION AND SYMPTOMS

Tuberculosis of the eyelids occurs as lupus, ulcers and generalized thickening of the lids. Lupus is usually associated with a similar condition of the skin of the face. It occurs most frequently between the ages of twenty-six and thirty years, and in Lundsgaard's experience (1) more females than males were affected. This condition is rather common in Europe, but is rarely seen in this country. The cervical and maxillary lymph nodes never suppurate in lupus. Lupus of the dermal surface of the eyelids may extend to the conjunctiva. When it is in this location it presents the same clinical picture as is seen elsewhere in the body.

Ulcers of the palpebral conjunctiva, when associated with enlargement of the preauricular or submaxillary lymph nodes, are usually due to tuberculosis (fig. 1). Parinaud's conjunctivitis produces similar symptoms. However, in tuberculosis, tubercle bacilli are often found in the secretion or scrapings from the ulcer. When bacilli appear in smears and animals inoculated with the secretions develop tuberculosis, the diagnosis is established. The microscopic examination of the tissue usually reveals the presence of a tuberculous structure. Tuberculous

ulcers of the conjunctiva may be primary, following the introduction of tubercle bacilli into the conjunctival sac. In our experience with animals, this condition occurred repeatedly after the conjunctiva had been lacerated, but it did not occur following the instillation of an emulsion of bacilli into the conjunctival sac in which the epithelium was intact. Conjunctival involvement also occurs as a secondary tuberculosis of endogenous origin. In our experiments, minute ulcers of the conjunctiva occurred as a secondary manifestation of tuberculosis in animals that had been inoculated (intracarotid) with tubercle bacilli. The bacilli had been carried to the conjunctiva by the blood.

Clinically, the first manifestation of tuberculosis in the conjunctiva is the presence of one or more rather pale, reddish-white nodules which are quite firm. These slowly increase in size, gradually break down and form ulcers which have a punched-out appearance and irregular edges. The ulcers are covered with a grayish-white or yellowish necrotic material. When this is removed, fine, pale granulations are noted in the base of the ulcer. The granulations do not bleed readily. A relatively narrow collar of induration surrounds the edge of the ulcer. The preauricular and cervical lymph nodes are invariably enlarged, and it is not uncommon for the preauricular node to suppurate and break down. Tubercle bacilli have been found in the pus discharging from these nodes, and animals inoculated with it usually develop the disease.

In experimental tuberculosis of the conjunctiva of the lids in rabbits, it is not uncommon for a fistula to form and perforate the skin surface of the lid, and the contents of the abscess to discharge externally. We have observed this occurrence many times.

The amount of destruction, produced by tuberculous ulcers, varies with the severity of the condition and with the effect of local and general treatment. If an arrest is effected, the diseased area is covered with scar tissue which results in a certain amount of deformity of the lid.

Tubercles in the bulbar conjunctiva occur as single or multiple nodules which resemble those just described. These may break down and produce characteristic tuberculous ulcers. When ulceration occurs, the cornea and sclera may become involved.

There has been a great deal of dispute among ophthalmologists and pediatricians regarding the etiology of phlyctenular conjunctivitis and keratitis. Some contend that the condition is always due to tuberculosis, while others believe that there are numerous other causes. Phlyctenulae of the conjunctiva and cornea are most frequently seen

in poorly nourished children, and it is more than probable that it is the inanition, which is associated with the tuberculosis, that is the underlying factor. Microscopically, phlyctenulae resemble tubercles, but tubercle bacilli are rarely found in them and may be the result of contamination.

Tuberculosis of the cornea is rarely primary. It does occur, however, following injuries with a coincident direct inoculation of tubercle bacilli into the corneal substance. Panas and Vassaux (2) succeeded in producing tuberculosis in the cornea of rabbits by direct inoculation, as early as 1886. We have also produced corneal ulcers and diffuse corneal infiltrations by inoculating suspensions of tubercle bacilli into the cornea (fig. 2). As a rule, when tuberculosis of the cornea exists, there is more or less inflammation of the iris and ciliary body; and, conversely, corneal tuberculosis is frequently secondary to tuberculosis of the uveal tract (iris, ciliary body and choroid). The conjunctiva and sclera are frequently involved to a varying degree when tuberculosis of the cornea exists. One or both eyes may be affected.

Tuberculosis occurs in the cornea as a parenchymatous keratitis, and is characterized by a more or less diffuse opacity of the cornea (fig. 3). As a rule the opacities vary in density and are often limited to one part of the cornea. They differ in this respect from the changes which occur in the cornea as a result of syphilis. Experimental tuberculosis of the cornea frequently resembles the keratitis which occurs in syphilis. Clinically, photophobia, lachrymation and a varying degree of visual impairment are complained of. The eyeball is usually congested. The cornea may be involved to a slight degree; and small points of white or yellowish infiltrates, confined to the middle and deeper layers, are often all that can be seen. These are isolated tubercles (fig. 4). Blood vessels in the cornea are usually a later manifestation of the disease, and are not found as frequently as they are in cases of syphilitic involvement. Parenchymatous keratitis, characterized by a diffuse opacity of the cornea and associated with only slight pericorneal congestion, is often due to tuberculosis. Grayish opacities in the cornea, which resemble cold mutton-fat in appearance, are suggestive of tuberculosis of this structure. Corneal tuberculosis is essentially chronic, and activity often covers a period of years. The degree of visual impairment depends upon the location and density of the corneal infiltration.

In some cases the opacities disappear entirely; in others, scars and blood vessels remain permanently and impair the vision to a varying degree.

Episcleritis and scleritis are often due to tuberculosis. In episcleritis a superficial, purplish, nodular swelling is noted between the sclera and the conjunctiva (fig. 5). As a rule, the condition is associated with only a slight amount of pain and photophobia. The congestion is confined to the anterior ciliary vessels, which are deeper than the conjunctival arteries and veins. This condition is usually chronic, and recurrences of inflammation over a period of years is the rule.

The sclera is often involved in tuberculosis of the uveal tract. Tuberculosis of the sclera is characterized by a thickening of the sclera with a deep purplish congestion surrounding the involved area. The condition is extremely chronic, and relapses are common. Photophobia, lachrymation and slight tenderness upon pressure are usually the only symptoms complained of. If the ciliary body and choroid are involved coincidentally, there is some impairment of vision.

Tuberculosis of the iris was first described by von Graefe as a granuloma, because sections of the iris which were examined microscopically resembled granulation tissue. It is found most often in youth, occurring usually between the ages of five and twenty-four years. The condition, however, has been reported as occurring in infants and old persons. Tuberculosis of the iris occurs in several forms: the most characteristic type is seen as miliary tubercles in the substance of the iris. Large solitary tubercles are often present, and it is frequently seen in the form of confluent or conglomerate tubercles.

A diffuse iritis without the formation of nodules is often due to tuberculosis. It is not always possible to distinguish this type, clinically, from other forms of chronic iritis. This variety of iris tuberculosis has been described as toxic, because of the difficulty of finding tubercle bacilli in sections of the iris which have been removed and examined microscopically.

The miliary type is characterized by the presence of small yellowish or grayish elevations on the iris surface (fig. 6), with more or less general thickening of the surrounding iris stroma. The blood vessels are only slightly distended in the beginning, and later in the disease this, as a rule, is not a prominent factor. This differentiates it from several of the other types of iritis. There is slight deepseated congestion of the vessels which surround the cornea (circumcorneal injection). Slight photophobia and pain are complained of. If the ciliary body is involved at the same time, there are minute deposits on the posterior surface of the cornea. These are often arranged in a triangular fashion, with the

base of the triangle downward. They are best seen with concentrated light and a corneal *loupe*. Frequently, the corneal substance is infiltrated with isolated collections of cells. This process continues over a variable period of time, and either undergoes resolution followed by scars and localized areas of atrophy in the iris, or progresses until the tubercles become confluent and result in large tuberculous masses (fig. 7). The condition may progress, and invade the ciliary body and the sclera at its junction with the cornea, and ultimately perforate the eyeball (fig. 8). This type of tuberculosis of the iris is usually associated with a generalized tuberculosis. These patients frequently die from a tuberculous meningitis.

It is not always easy to differentiate tuberculosis of the iris from syphilis and focal infection. These conditions must be borne in mind, and their presence eliminated before making a positive diagnosis.

Tuberculosis of the ciliary body is difficult to differentiate from other inflammations of this portion of the eye. When it exists in this structure *per se*, it is impossible for one not familiar with diseases of the eye to make a positive diagnosis of tuberculosis of the ciliary body.

Tuberculosis occurs rather frequently in the choroid and retina coincidentally. The only symptoms, however, that might excite the internist's suspicion that tuberculosis exists in these structures is a change in the visual acuity. Pain, and symptoms other than these are not complained of. Unless the internist is familiar with the use of the ophthalmoscope, there is no possible way for him to determine the cause of the loss of vision. It is because this condition is not uncommon, that attention should be directed to it. Internists frequently are skeptical about accepting the suggestion that an obscure intraocular disease may be tuberculous. This is especially true if the patient is robust and apparently in good health, which is frequently the case in chronic types.

Miliary tubercles of the choroid are frequently seen in early tuberculous meningitis, especially in children; and the nature of an obscure meningeal condition can frequently be determined by ophthalmoscopic examination when tubercles are found in the choroid (fig. 9). Small yellowish tubercles which vary in number can be readily seen in the choroid in these cases. This is usually a manifestation of an acute generalized miliary tuberculosis.

Jäger was the first to clearly describe disseminated choroidal tuberculosis. This condition is usually chronic. It destroys the choroid and deeper layers of the retina. In the terminal stage scars and pigmentary changes are seen in the fundus of the eye.

Tuberculosis of the retina is a rare disease. Its true nature has been understood for only a short period of time. The condition occurs as a vasculitis or a perivasculitis of the retinal vessels, or as small white areas in the retina which resemble the exudates found in albuminuric retinitis. The vessels, when involved, frequently rupture, and hemorrhages into the retina and vitreous occur. Hemorrhages absorb slowly and are frequently replaced by fine strands of scar tissue (*retinitis proliferans*) (fig. 10). The hemorrhages are usually recurrent. Following hemorrhages, the vision is markedly impaired. The amount of impairment depends upon the extent and location of the bleeding. Both eyes are usually involved in this process, and the prognosis regarding vision is poor. This type of tuberculosis, as a rule, is of the chronic variety, and it is frequently seen in persons with latent tuberculosis. It is often impossible to find the primary seat of the disease, as these patients appear to be perfectly healthy. Cases of this type have been reported in persons who were apparently well at the time of the first attack, but who subsequently developed other manifestations of tuberculosis elsewhere in the body during later periods of activity in the eye (pleurisy or active pulmonary tuberculosis.) Tuberculous masses may develop in the retina and be mistaken for intraocular tumors. They are usually associated with tuberculosis of the optic nerve or ciliary body and are only diagnosed after histological examination of the eye.

Tubercles of various types in the optic nerve have been described. The symptoms of this condition vary widely and depend upon the amount of involvement. A change in vision is the most prominent symptom. Early enucleation of the eyeball is advisable if the involvement is extensive or if the condition fails to yield to treatment. There is great danger of extension of the process when it is in this location, because the sheaths covering the optic nerve are directly continuous with the meninges of the brain.

The lachrymal duct and sac are frequently involved in a tuberculous process. The symptoms are similar to dacryocystitis due to other causes. Tuberculosis in this location is often complicated by fistulae which communicate externally with the skin. Often a secondary purulent dacryocystitis is engrafted on a tuberculous process in the sac. Tuberculosis of the nose is frequently found associated with this condition. The condition is usually unilateral, but both sacs may be involved. As a rule, the true nature of the sac condition is not discovered until sections of the extirpated sac are studied histologically. Epiphora (lachry-

mation) and pus in the lachrymal sac, which can be squeezed out by applying pressure over this structure, are the chief symptoms complained of.

The orbit has been reported as the seat of ocular tuberculosis, but this is not very common. Here the process is manifested by a diffuse low-grade inflammation in the orbital tissue. When the involvement occurs in back of the eyeball, the globe may be pushed forward and the symptoms of a retrobulbar tumor are present. The lymph nodes in the neck and in front of the ear are usually enlarged, and should excite suspicion as to the nature of the condition. Microscopic examination of the tissue and animal inoculation with secretions from the orbit, if present, will establish the diagnosis. It is difficult to make a differential diagnosis between tuberculous and malignant tumors in this location from the symptoms alone.

THE COURSE OF TUBERCULOSIS OF THE EYE

In the acute type of intraocular tuberculosis, active tuberculosis is usually found elsewhere in the body and the ocular condition is due to the presence of living tubercle bacilli. If the patient lives for a long enough period after the eye condition develops, a destructive ocular process will be observed. When the lesions are inside the eye, the involvement rapidly progresses until the eyeball is entirely destroyed, or until it ruptures and its contents are expelled. The eyeball may be converted into a caseous mass, which will necessitate removal. Patients with this type of ocular tuberculosis frequently die before the eye lesions have run their full course.

The chronic types are much slower in their development and are frequently confined to one structure. They are usually found in persons who have a low-grade chronic or a latent tuberculosis elsewhere in the body. Often the primary focus is never detected in the latter type. The lesions in this variety are frequently active for months or even years, and recurrences at varying intervals are the rule. Edward Jackson of Denver states that, as long as the patient lives, one is never certain that there will not be a recurrence of the activity in an eye which has apparently recovered from a tuberculous process.

DIAGNOSTIC METHODS

Under this heading an attempt will be made to present the subject to the internist from the ophthalmologist's point of view. In the past

we have been at a loss to determine whether these cases are within the province of the internist or of the ophthalmologist. At present it is agreed that the responsibility must be shared by both. No two branches of medicine require more concentrated effort and coöperation between internist and ophthalmologist than the diagnosis and treatment of cases of tuberculosis of the eye, and a satisfactory plan of coöperation can be carried out in the following manner: The cases are usually seen first by the ophthalmologist, and it becomes his duty to begin the investigation of the cause of the eye condition. When tuberculosis of the eye is suspected, it should be determined, first, whether the patient has an active focus of tuberculosis elsewhere in the body. To decide this, the patient should be referred to a competent internist for careful investigation. The thorax should be examined carefully, and chest roentgenographs made and reported on by a competent roentgenologist. The tonsils and cervical lymph nodes should be examined, to determine whether a scrofulosis of a tuberculous nature exists. If the temperature, taken over a period of two or three days, the physical signs, and other evidences of tuberculosis are negative, the use of tuberculin should be considered. It is a serious mistake on the part of an ophthalmologist to use tuberculin without first having the patient undergo the foregoing complete examination.

Old tuberculin is to be preferred for diagnostic purposes. The concentration of the first dose should be determined by the ophthalmologist, because the reaction of the foci in various parts of the eye differ markedly. Reactions in foci of the sclera and cornea do not involve hazards to visual acuity, while the retina and choroid reactions may permanently impair visual function. The dose, therefore, must be graduated in accordance with the type of lesion at hand. The usual primary dose of O.T., when used for diagnostic purposes in cases of ocular tuberculosis other than that involving the retina and choroid, is usually 0.1 mgm., injected subcutaneously. The Pirquet, Moro and Calmette tests are valueless as diagnostic tests in eye tuberculosis. The eye should be examined, preferably eight hours after the inoculation and again twenty-four hours after. Evidences of constitutional and local reactions should be watched for. If there is no reaction after forty-eight hours, a second injection of 1 mgm. should be given. If this proves negative, a third dose of 3 mgm. should be given. If this is also negative, 5 mgm. are administered forty-eight hours after the third dose. If no reaction occurs following a dose of 5 mgm., one is reason-

ably certain that the lesion is nontuberculous in nature. Proportionately smaller doses should be given to children.

The ophthalmologist divides reactions to tuberculin into three types: namely, *local*, which manifest themselves at the site of injection by the presence of pain, redness, swelling and tenderness, and occasionally slight enlargement of the regional lymph nodes; *constitutional*, manifested by headaches, pain in the joints, backache, malaise, weakness, and in some cases nausea and vomiting; and *focal*, occurring in the eye and manifested by increased injection, fresh exudates and hemorrhages. It is believed by most oculists that the presence of a focal reaction in the eye is a positive means of diagnosing tuberculosis. General and local reactions only prove that the patient is sensitive to tuberculin. It is desirable to obtain as slight a focal reaction as is consistent with making a diagnosis. Severe reactions frequently do permanent damage.

When active tuberculosis exists elsewhere in the body, it becomes the internist's duty to determine the advisability of using tuberculin as a diagnostic agent. He should determine the dose to be used and the frequency with which it should be administered. The oculist, in this case, should carefully watch the eye for focal reactions. When it is inadvisable to use tuberculin, because of the danger of dissemination, the diagnosis of ocular tuberculosis must be made by exclusion and the clinical manifestations in the eye. In addition to tuberculin, smears and material obtained by curetting should be examined microscopically and inoculated into animals, when possible. When animals, inoculated with secretions or tissue from the eye, develop tuberculosis, the diagnosis is established. It is always advisable to examine suspected tissues under the microscope as well, when this can be done.

TREATMENT OF TUBERCULOSIS OF THE EYE

Treatment should consist of hygienic measures, tuberculin and local therapy. It is essential, in treating tuberculosis of the eye, that the same coöperation between internist and ophthalomologist be observed as in the diagnosis. When the slightest evidence of tuberculosis elsewhere in the body exists, the internist should supervise the general hygienic treatment, as well as the dosage of tuberculin and the frequency of its administration. The ophthalmologist should carefully watch the eyes for a focal reaction. If in his judgment the reactions are too severe, he should advise the internist to diminish the dose. The ophthalmologist should treat the local eye condition symptomatically.

It is just as essential in ocular tuberculosis, as in tuberculosis elsewhere in the body, that the patient's hygienic condition be of the best. He should spend as much time out of doors as is possible, and in most instances it is advisable for him to abstain from all possible means of eyestrain. When the uveal tract is involved, atropin should be used diligently.

It is generally conceded by ophthalmologists that tuberculin is of great value in the treatment of ocular tuberculosis when it is used intelligently. The careless and unintelligent use of tuberculin has done much harm and has brought this valuable therapeutic agent into unjust disrepute as far as eye therapy is concerned.

While it is essential that the internist supervise the size of the dose of tuberculin as far as the patient's general condition is concerned, it is equally as important that he should consult the oculist regarding the pathological nature of the eye lesion, and what to expect if a severe focal reaction follows the subcutaneous administration of tuberculin. In lesions of the retinal vessels, reactions are followed by exudates and hemorrhages of varying degree. If the hemorrhages are severe, the patient's vision might be permanently impaired or entirely lost following the use of tuberculin. This eye condition requires very minute doses. Reactions have been reported after the administration of as small a dose as 0.0002 mgm. In retinal tuberculosis the initial dose of tuberculin should be 0.00005 mgm., and the dose doubled at weekly intervals. The patient should be carefully watched for a focal reaction. If this occurs the dose should be diminished and kept below the reaction point.

In tuberculosis of other portions of the eye, the dose should be kept just below the reaction point. The amount which produces a reaction is usually determined by the diagnostic dose.

It is unfortunate that many persons look upon tuberculin as a specific remedy for tuberculosis of the eye. For this reason its value has been overestimated in some instances and condemned as worthless in others. The majority of competent ophthalmologists feel certain that they have seen marked improvement in cases of ocular tuberculosis following the administration of tuberculin subcutaneously.

The physiological action of tuberculin differs in different persons, and different preparations produce different reactions. For this reason an ironclad rule cannot be laid down for its administration. However, the bacillary emulsion (B.E.) is to be preferred for therapeutic purposes.

It is wise to start with minute doses, and gradually increase the strength. Each patient must be studied separately, and the dose regulated according to his peculiar needs. It should be administered at regular intervals. It is the practice of some physicians to administer tuberculin every four days, while others recommend intervals of seven to ten days. At the Knapp Memorial Hospital, New York City, an initial dose of 0.0001 mgm. is given, and an increase of 0.0001 mgm. with each subsequent dose at four-day intervals, until 0.001 mgm. is reached. Then 0.001 mgm. is added to the dose at the same intervals, until 0.01 mgm. is given. The doses are then administered at weekly intervals: 0.01 mgm. per dose until 0.1 mgm. is reached. The interval is then lengthened to two weeks and 0.1 mgm. is added to each dose. When 1 mgm. has been attained the interval is lengthened to one month.

It is a good rule to use tuberculin for several months after all evidence of activity has disappeared. If there are recurrences of activity, the use of tuberculin should be resumed cautiously.

Favorable results have been reported in the treatment of some forms of tuberculosis of the eye from the use of radium preparations and the roentgen rays. Their value, however, is questionable. The Finsen light has been used with good results in the treatment of conjunctival tuberculosis and lupus. Exposure of conjunctival tubercles to graded doses of direct sunlight caused them to disappear in George F. Libby's case (3). Numerous other methods of treatment have been reported on favorably, but they are of questionable value.

Appreciation is due Dr. H. J. Corper for valuable data used in this paper.

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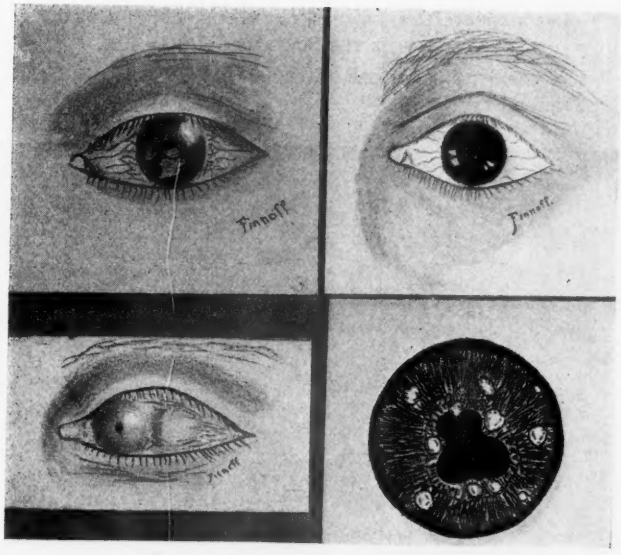
FIG. 1 FIG. 2

FIG. 1. TUBERCULOUS ULCER OF THE PALPEBRAL CONJUNCTIVA, WITH ENLARGEMENT OF THE PREAURICULAR LYMPH NODE

FIG. 2. PHOTOGRAPH OF ULCER OF THE CORNEA WITH HYPOPYON

Eye taken from the rabbit ten days after the injection of tubercle bacilli into the corneal substance.

3 4



5 6

FIG. 3. PARENCHYMATOUS KERATITIS DUE TO TUBERCULOSIS

FIG. 4. SMALL ISOLATED TUBERCULOUS EXUDATES IN THE CORNEA

FIG. 5. TUBERCULOUS EPISCLERITIS

FIG. 6. MILIARY TUBERCLES OF THE IRIS

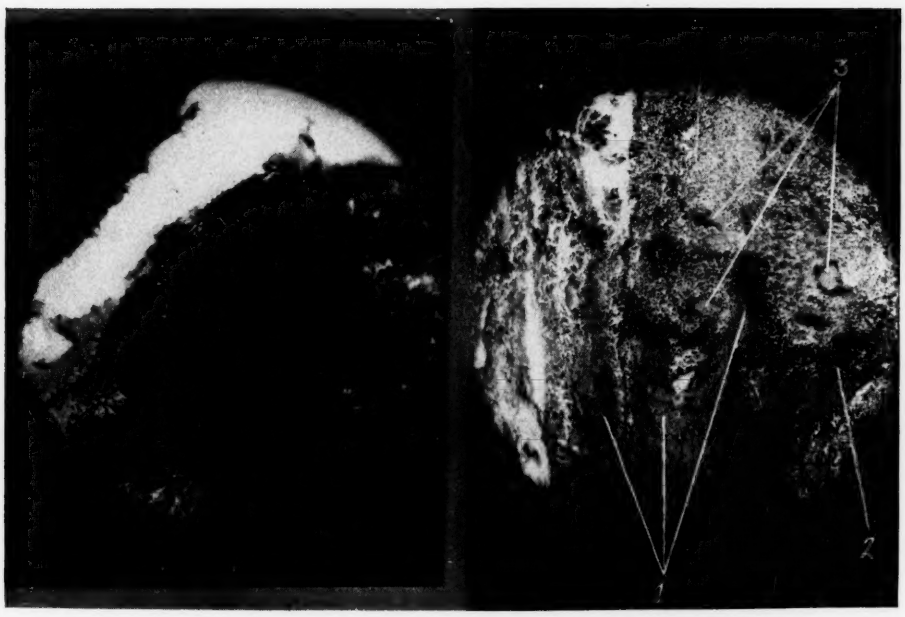


FIG. 7 FIG. 8

FIG. 7. PHOTOMICROGRAPH FROM RABBIT'S EYE SHOWING CONGLOMERATE TUBERCLE OF THE IRIS AND LARGE CASEOUS MASS

FIG. 8. PHOTOMICROGRAPH: TUBERCULOSIS OF THE IRIS AND CILIARY BODY, WITH PERFORATION OF THE SCLERA IN HUMAN EYE

1, Sclera; *2*, ciliary body; *3*, giant cells; *4*, endothelial cells and small round cells which have entirely replaced the sclera.

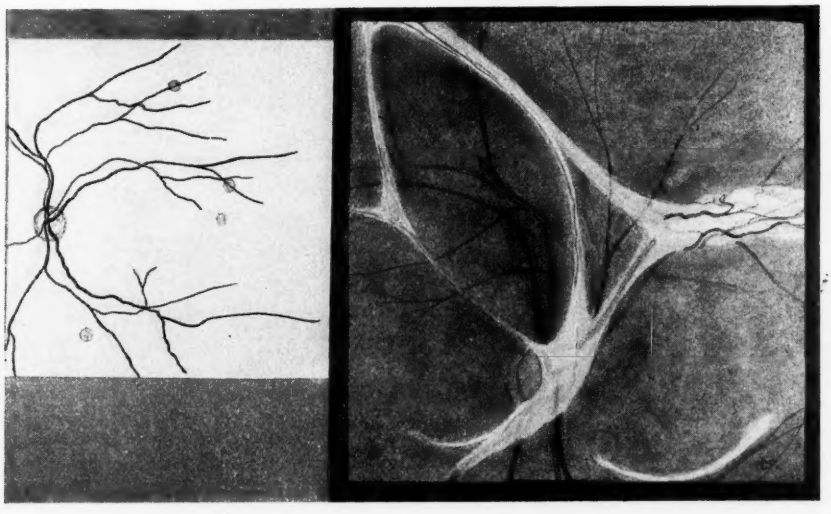


FIG. 9 FIG. 10

FIG. 9. MILIARY TUBERCLES IN THE CHOROID AS SEEN THROUGH THE OPHTHALMOSCOPE

FIG. 10. BANDS OF *Retinitis Proliferans* AS SEEN TWO AND ONE-HALF YEARS AFTER ONSET OF TUBERCULOSIS OF THE RETINA

Ophthalmoscopic appearance

TUBERCULOSIS OF THE EYE AND ITS TREATMENT

A REVIEW OF RECENT LITERATURE

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Prior to 1915 almost all forms of tuberculous lesions of the eye had been described; even as early as 1818 tuberculosis of the choroid was reported. Since 1915 there have been a great many articles and discussions, constituting a revival of interest in the subject, with seemingly more attention given to tuberculin diagnosis and treatment than to clinical reports.

Tuberculosis of the conjunctiva: This condition seems to have been found rather frequently, as Libby, Patterson, Weeks, Pusey, Coover, Friede, Cohen, Bayer and Guzman have written upon the subject. The clinical appearance has been chiefly of the ulcerative type, and treatment has been notable for its variety. Libby used heliotherapy and tuberculin, while Patterson used trichloracetic acid and tuberculin. As to Parinaud's conjunctivitis, Verhoeff claims it to be nontuberculous and supports his claims by some very excellent work. Casey Wood calls attention to Eyres's five types of involvement of the conjunctiva, namely, (1) the ulcerative form, (2) miliary tubercle, (3) hypertrophic granulations, (4) lupus, and (5) pedunculated tumor. Of the cases recently reported, the ulcerated type is the one that has been most frequently seen.

Phlyctenular conjunctivitis: Goldbach, in his series of 100 cases, showed that nearly all had evidences of a tuberculous infection, and believed the condition at least allied with it, while Goldenburg reported 500 cases in which there was no evidence of tuberculosis, but nearly all a result of errors in diet. Others have discussed the question but no definite conclusion has been reached. Many believe that in the very refractory cases the therapeutic use of tuberculin is of definite benefit.

Tuberculosis involving lachrymal sac: There seems to be only one article on this subject, that of Polyák, in which he claims that out of 400 cases of dacrocystitis, 52 or 13 per cent were complicated by tuber-

culous infection. This very important statement merits further study by careful examination of all extirpated lachrymal sacs.

Strabismus: Rivers claims that squint is an indication of pulmonary tuberculosis and *his* figures confirm it. This seems so contrary to our experience that a very large statistical study should be made before accepting his statement.

Corneal involvement: There are the two conditions spoken of as being at least associated with tuberculous infection, namely, *keratitis profunda* and *phlyctenular keratitis*. Many cases of the parenchymatous keratitis or keratitis profunda have been reported and have, as a rule, shown marked improvement under tuberculin therapy. The required dose has at times been quite large before beneficial results were apparent. Ellis and Gay make the absurd statement that practically all maculae and nebulae of the cornea are tuberculous in origin and should be treated by local instillations of tuberculin. Several have called attention to cases of keratitis with positive Wassermann which fail to improve under antiluetic treatment, but do show marked improvement when tuberculin is given in conjunction with the antiluetic treatment, showing the mixed character of the infection. Possibly one of the most significant articles on corneal conditions is one by Koeppe, in which he records the healing of a rodent ulcer of the cornea by the use of tuberculin.

Tuberculous iritis: Mulcahy and Ewing report a child of three years of age as having had a tuberculoma of the iris, which reacted focally to 0.25 mgm. O. T. Later the child died of bronchopneumonia and the case was autopsied, confirming the clinical diagnosis, although no bacilli were found. Thompson reports tuberculoma of the iris, which improved under tuberculin in dosage of 7 mgm. O.T. every ten days. Finnoff records a case with nodule on the iris and keratitis profunda as being tuberculous. Stark reports three cases with tuberculous iritis, and he believes that this lesion is the most frequent manifestation of a local tuberculosis of the eye. Hessberg claims that 50 per cent of iritis cases are due to tuberculosis.

The greatest help in diagnosis is the focal reaction following the use of tuberculin. It seems also that the greater number of authors depend upon the use of tuberculin for treatment, although Tooke used iodoform introduced into the anterior chamber, but without result, and Bock claims a cure by use of the "immune bodies" of Karl Spengler.

Tuberculous choroiditis: Randolph and Schmeisser studied two cases of miliary tubercles of the choroid, both ophthalmoscopically and

microscopically. They believed it usually present in the terminal stages of general miliary tuberculosis. Sidney Stephenson speaks of it as a means to a general diagnosis.

A great many have reported cases of uveitis and choroiditis which were diagnosed by focal reactions following tuberculin, and most of these improved after tuberculin therapy. H. Snellen, however, gives an interesting report of an intraocular growth, diagnosed clinically as sarcoma of the choroid, which, microscopically, proved to be tuberculoma. It is the belief of most authors that tubercle bacilli are very frequently the cause of acute and chronic choroiditis.

Retinal tuberculosis: This has been the subject of many articles, among those contributing being Jackson, Finnoff, Wilmer and Thompson. Recurring haemorrhages with perivasculitis and *retinitis proliferans* seem to be the chief characteristics. Jackson lays stress upon the cases of recurring haemorrhages as those liable to develop into *retinitis proliferans*. Here again tuberculin is used in treatment, although the dose should be very minute, it being often dangerous to give a diagnostic dose, but increasing therapeutic doses may be given until a focal reaction is obtained.

Optic nerve tuberculosis: Sidler has written on tuberculosis of the optic nerve. Bär reports a case of choked disc resulting from tuberculosis of the nerve, while Oloff reports a case of sudden blindness which gave a positive reaction to O. T. and improved under tuberculin therapy.

Tuberculosis of sclera: Knapp believes that a scleral ectasia in the anterior segment, combined with intraocular disease, should be regarded with suspicion and always have a thorough investigation as to its being of tuberculous origin. He reports two cases. While the question of the frequency of tubercle bacilli, as a factor in episcleritis and scleritis, has been much discussed in the past it does not seem to have occupied a leading part in recent literature.

General pathology: Von Hippel in a long and well illustrated article reported his studies on the histological similarity between tuberculous eye lesions and those resulting from sympathetic ophthalmia, and while he has never demonstrated tubercle bacilli in the nodules of sympathetic lesions he is unable from the histological standpoint to make a differential diagnosis between them. J. Meller has also studied a case of sympathetic ophthalmia, where he was able to examine both eyes microscopically, in which he arrived at very much the same conclusions. This very careful study, combined with the reports of cure of sympathetic ophthal-

mia with tuberculin, seems to be very significant and worthy of very careful investigation.

Treatment of tuberculous lesions: While most authors seem to put considerable faith in tuberculin treatment combined with general hygienic measures, the use of subcutaneous injections of milk in these conditions has received marked attention abroad. Kraupa in a recent article has taken this question up rather completely, and believes that, while one may get similar results by other lines of treatment, especially tuberculin, he can accomplish the same improvement in much shorter time by one milk injection than by long and continued injections of tuberculin. He thinks that all the various tissues involved do well under this form of treatment if their origin be tuberculous. Saupe claims good results from partial antigens according to Deycke-Much; Hessberg believes krysolgan useful in absorbing exudates; and Cohn reports good results from instillations of sodium cinnamylicum.

Diagnostic use of tuberculin: Weeks recommends 0.5 mgm. in children and 1 mgm. in adults as initial dose, going up to 3 mgm., while others recommend up to 5 mgm. Such doses may be used in corneal, scleral or iritic conditions, but in those lesions involving structures such as the retina and the choroid, near the macula or the nerve head, very small therapeutic doses should be used, gradually increasing until a focal reaction is obtained.

Therapeutic use of tuberculin: All seem of one accord that in the more or less chronic stages its use is alway beneficial, while in some of the very acute conditions the indications for its use are not so positive. The size of the dose varies greatly, both as to the preparation used and tissue involved, corneal conditions requiring much larger doses than those of the vascular coats or lesions situated close to the macula. But practically all testify to its utility when properly used.

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STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS

XXV. TISSUE CHANGES PRODUCED BY THE ACTION OF THE LIPINS[1] OF TUBERCLE, GRASS AND COLON BACILLI, AND OF LIVER

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Auclair (1) reported that he had produced caseation-necrosis in guinea pigs and rabbits by subcutaneous and intratracheal injections of lipoidal materials, extracted from human tubercle bacilli with ether and chloroform. In a later publication (2) he referred to his experiments with ether and chloroform extracts, and stated that xylene and benzene extractions yielded substances with similar capacity to produce caseation. In a still later work (3) he stated that he produced a typical caseous pneumonia in the lungs of a rabbit by intratracheal injections of the ether extract of tubercle bacilli.

Morse and Stott (4) produced a fibroblastic reaction of the epithelioid type with numerous typical cellular tubercles and characteristic giant cells in rabbits, by means of subcutaneous, intraperitoneal and intravenous injections of the alcohol extract of tubercle bacilli. The ether extract they found to be inert. In no case did they observe caseation. They concluded that the tubercle bacillus produces a characteristic microscopic lesion, not because it is a living particulate protein but because its waxy substances act as a peculiar type of foreign body.

As far as we can determine there has been no experimental work done on the tissue changes produced by the lipins of nonpathogenic microorganisms. Gaehlinger and Tilmant (5) made experiments with "liver lipoids," and claimed that there exist in the liver lipoids some acid-resisting fatty acids capable of causing tubercles with caseation when these lipins are injected subcutaneously. This property suggests that

[1] In this article the word *lipins* is used in its comprehensive sense to include simple fats, lipoids, fatty acids and related substances.

the hepatic lipins must be similar to the caseifying poison extracted from tubercle bacilli by Auclair, which he designated as *ethero-bacilline*.

We have attempted to repeat the work of Gaehlinger and Tilmant and, in addition, have made an effort to determine the tissue reactions to similar extracts of grass bacilli and colon bacilli, and to compare them with the reactions to corresponding preparations from tubercle bacilli.

PREPARATION OF MATERIALS

Two methods of extracting the lipoids from the bacilli were used. One is essentially similar to that employed by Morse and Stott, except that after the hot alcohol extraction a hot chloroform extraction was made; the other method is different, and was suggested by Dr. E. R. Long. In subsequent references we shall designate the first method as the Morse method, and the second as the Long method.

Morse's Method: Fifty grams of dry tubercle bacilli were put into a flask, 1.5 litres of ether were added, and the flask allowed to remain in a refrigerator over night. The ether was then poured off, passed through a hard filter paper, then through a fine Berkefeld filter, and evaporated practically to dryness and stored in sterile bottles. To the bacilli remaining in the flask 1.5 litres of 95 per cent alcohol were added, and the contents boiled under a reflux condenser for two hours. The alcohol was poured off, filtered hot through a hard filter paper, then through a Berkefeld filter, and evaporated and stored as was the ether extract. To the bacilli remaining in the flask was added 1.5 litres of chloroform, and an extraction made exactly as with the alcohol. The ether-extracted lipin was waxy, solid and dark red. The alcohol-extracted lipin was like semiliquid honey, with at first a honey-like odor, which changed on standing to an indescribable "musty" odor. The chloroform-extracted lipin was a glassy, waxy material, caramel colored.

Long's Method: Three hundred grams of dried tubercle bacilli were put into a large flask with three litres of 95 per cent alcohol and allowed to stand for three days. The alcohol was then poured off, distilled to about 400 cc. volume, filtered through a hard filter paper and then through a Berkefeld filter,[2] poured into a large evaporating dish, evaporated almost to dryness on a water bath, and the dish set aside as a

[2] The passage through the Berkefeld filter was to remove any solid bacillary substance, for extracts merely filtered through paper commonly contain many dead bacilli, which might possibly be responsible for the tissue reactions observed.

receptacle for the hot alcohol extractions which followed. As before, three litres of 95 per cent alcohol were poured into the flask, which was then boiled under a reflux condenser for two hours, and the alcohol treated as before. Then two extractions were made in the same manner with absolute alcohol, the same evaporating dish being used in the final concentration in each case. The bacilli remaining in the flask were then subjected to two hot toluene extractions, using three litres in each extraction, and employing exactly similar technique, another evaporating dish, however, being used for the final concentrations.

Thus we had one large evaporating dish containing all the lipins extracted by the alcohol, and another containing the lipins extracted by toluene. From each of these dishes was made an ether extraction, and the ether used in making the extraction from one dish was mixed with that used in the other dish, then passed through a Berkefeld filter, evaporated to dryness, and stored in a sterile bottle. A hot chloroform extraction and then a hot absolute alcohol extraction were made from the lipins remaining in the two original dishes, the same methods being employed as in the first extraction with ether. All of the lipins went into solution in one solvent or the other, no residue remaining in the dishes. By this method a far larger percentage of lipins was extracted from the bacilli than by Morse's method.

The ether-extracted lipin fraction closely resembled that obtained with ether by the Morse method, but was somewhat darker red in color, with an odor suggesting chocolate. The chloroform-extracted lipin resembled crushed pine rosin, but was rather softer and its fracture was not so glassy. Its odor reminded one of honey. The alcohol-extracted lipin also had a honey-like odor, and in general resembled that extracted by alcohol with the Morse method.

Even after the vigorous measures employed in the Long method of extraction, some lipin yet remained in the bacilli. Twenty-five grams of the whitish, friable cake constituting the residue were put into a two-litre flask, a litre of chloroform was added, and the flask boiled an hour under the reflux condenser. The chloroform was poured off, a litre of toluene was added, and the contents refluxed another hour. The chloroform extracted very little lipin, and the toluene practically none. The bacilli were then assumed to be almost fat-free although many are still acid-fast, but Long (6) has recently shown that even such thoroughly extracted bacilli will still yield a considerable amount of lipoidal material after disintegration with acids.

Grass Bacilli: These were grown on glycerine broth, pH 7, in 500 cc. flasks at 37°C. At the end of about three weeks, when there was a heavy growth, the flask of broth with bacilli was poured into a 1000 cc. graduate and allowed to stand until the bacilli had settled, some sinking to the bottom and others floating on top of the broth. The intermediate broth was pipetted off and the bacilli were left with a small amount of broth; these were washed several times with distilled water to free them from broth and then with 95 per cent alcohol to free them from water. We obtained about 50 cc. of wet grass bacilli by this method, and to this amount we added two litres of cold ether and allowed this to stand over night in the ice-box, then filtered, evaporated and bottled this extract. To the residue we added two litres of alcohol and refluxed for three hours, then filtered, distilled, evaporated and bottled as before. The residue was then boiled under a reflux condenser with two litres of chloroform for three hours, filtered, evaporated and bottled in the same manner as the ether extract. All of the grass bacillus lipins were of a waxy consistency and had a light canary-yellow color.

The residue of grass bacilli was then refluxed three hours with absolute alcohol and again with toluene; but no lipin was removed; so the bacilli were considered fat-free and were dried, bottled and labelled as such.

Colon Bacilli: These bacilli were grown in the same manner as the grass bacilli, but it was necessary to centrifugate the broth with the growth for at least one-half hour at high speed to obtain the bacilli. The broth above the bacilli, which were thus thrown down, was pipetted off and the bacilli were washed in water and alcohol as the grass bacilli were. These bacilli were then extracted with cold ether, hot alcohol and chloroform, but the lipin obtained in each extraction was so small in amount that we combined all the extracted lipins and placed in the same bottle which was labelled *Colon Bacillus Lipins.* This was dark greenish-black in color and formed a rather solid mass with very little odor. The residue of bacilli was treated with absolute alcohol and toluene as with the grass bacilli extraction, and was then bottled, sealed and labelled *Fat-free Colon Bacilli.*

Liver: Two pounds of beef liver were ground in a meat chopper and allowed to stand over night in three litres of 95 per cent alcohol; this was filtered the next day through filter paper and then through a Berkefeld filter, and then placed in a clean bottle. To the residue three more litres of 95 per cent alcohol were added, and this was allowed to stand over night, to be filtered the next day as before. The two filtrates were

mixed and all but about 50 cc. of the alcohol distilled off; this was then evaporated over a water-bath, leaving a dark brownish, greasy mass of lipins, which gave off an indescribably nauseating odor with a suggestion of caramel. This was placed in a clean bottle, sealed and labelled *Cold Alcohol Extract of Liver*. To the residue from the above filtration three litres of cold ether were added and this was allowed to stand in the ice-box over night. This was then filtered and evaporated as above, leaving a golden yellow, unctuous mass which gave off a nauseating, acrid odor. This was bottled, sealed and labelled *Cold Ether Extract of Liver*. To the remaining residue was then added three litres of 95 per cent alcohol and this was boiled for three hours under a reflux condenser, after which it was filtered hot, and the alcohol distilled off and evaporated as the cold alcohol extract had been, leaving a material of golden brown color, soft and granular. This was bottled, sealed and labelled *Hot Alcohol Extract of Liver*. The residue was then refluxed with three litres of chloroform for three hours, after which it was filtered and evaporated as the other solvents had been; but the lipins taken out by this solvent were scanty and of a brownish color tinged with green. This was bottled, sealed and labelled *Chloroform Extract of Liver*. The fat-free residue was then discarded.

METHOD OF INJECTION

One gram of the desired lipin mixture, or fat-free bacilli, was carefully weighed and ground in a mortar with 3 to 5 cc. of 1 per cent sodium stearate solution, to which distilled water was added slowly to 100 cc., until all the material was emulsified. All emulsions thus contained 1 per cent of their respective lipins or fat-free bacilli. These emulsions were preserved in clean bottles, which were plugged with cotton and placed in the autoclave for twenty minutes at fifteen pounds pressure, then removed and the cotton plugs replaced by sterile corks.

Guinea pigs were used in all our experiments, and two injections of 1 cc. were made on each, one subcutaneous injection in the right groin and one intrapulmonary injection between the fourth and fifth right ribs. The animals were all shaved over the area where injection was to be made and washed with alcohol to avoid infection. At least three animals were injected with every emulsion, and observed every day for two weeks. Then they were killed by chloroform or illuminating gas and autopsied.

RESULTS OF INJECTION OF TUBERCLE BACILLUS LIPINS

1. Injection of alcohol-extracted lipins: The lipins extracted with alcohol by both methods produced, subcutaneously, at the site of inoculation, a reddened swelling, followed by subsidence of the swelling and the formation of a small hard nodule, which persisted in only two cases.

Histologically, lipins extracted by both methods produced some endothelial and lymphoid hyperplasia in the regional lymph nodes, and in the subcutaneous tissues a fibroblastic reaction of the epithelioid type. Giant cells, both those with the nuclei arranged in the form of a horseshoe and those with the nuclei clumped toward the centre, were present in the epithelioid tissue produced by lipins of both methods, but were somewhat more numerous in the lesions produced by the Morse-method lipins. Except in one case, in which consolidation of the middle lobe of the right lung occurred, no changes beyond atelectasis were observed in the lungs.

2. Injection of ether-extracted lipins: Subcutaneous injection of lipins extracted by the Long method produced a local swelling and reddening, followed by a small nodule, which regressed to a size barely palpable at the time of autopsy. The inguinal lymph nodes were enlarged. Intrapulmonary injection of this material produced no definite gross lesions.

Lipins extracted by the Morse method produced, subcutaneously, a more severe reaction. The nodules following the primary swelling were much larger than those produced by the Long-method lipins, and one showed a centre of caseoid material. The regional lymph nodes were considerably enlarged. Gross lesions were produced in the lung also, definite consolidations, hard and showing grayish islands. The mediastinal lymph nodes were greatly enlarged, in one case some of them reaching a diameter of 1 cm.

Histological study of tissues taken from guinea pigs injected with Long-method lipins showed no deviation from the normal in the subcutaneous tissue, though the inguinal lymph nodes were somewhat hyperplastic. In the lung, in addition to atelectasis, there was a fibrosis and edema around the larger vessels and bronchi. A great increase in the lymphocytic elements was apparent, in some cases the cells having eroded through the bronchial wall, destroying the continuity of the muscle and cartilage layers, and raising the mucosa adjacent to the break by reason of the large numbers of cells which had wandered in. In one case, in which the material failed to enter the lung, the pleura was

markedly fibrotic and thickened. Nowhere was there any caseation, fibroblastic reaction of epithelioid type, tubercles, or giant cells.

Microscopically, as grossly, the lesions induced by the Morse-method lipins were more severe than those induced by those of the Long method. In the subcutaneous tissue there was a marked fibroblastic reaction of the epithelioid type, the epithelioid tissue containing a few giant cells of both of the types previously mentioned. The substance resembling caseation-necrosis to the naked eye was seen to be merely pus, contained in a large fibrous-walled abscess. Other smaller abscesses, not noted in the gross examination, were also seen in other sections. Some of these were walled off by a fibrous tissue not of epithelioid nature, while some were surrounded by epithelioid tissue containing giant cells. Some sections contained a granulation tissue of definite epithelioid type. The muscle fibres subjacent to the lesions were swollen, vacuolated and hyalinized, and widely separated by the epithelioid tissue which had infiltrated between them.

This same type of tissue was also very abundant in some of the lymph nodes, the normal architecture being obliterated by it. A few giant cells were present. The capsules were greatly thickened.

The lungs injected with the Morse-method lipins showed a beautiful epithelioid type of reaction, in some sections its distribution being general throughout, in others not so widespread. The smaller bronchi and some of the larger ones were full of desquamated epithelial cells and fibrin. The same proliferation of the lymphoid elements produced by the Long-method lipins was observed, and the same erosion through the bronchial walls. The blood vessel intima was somewhat proliferated. On the pleura were both the simple fibroblastic reaction, epithelioid in type, and granulation tissue of similar type. A few giant cells of both types were present, but there was neither caseation nor tubercles.

3. Injection of chloroform-extracted lipins: Subcutaneous lesions produced by lipins extracted by both methods were practically alike. After a primary edematous swelling, a hard nodule appeared early and attained considerable size, in one case being nearly 2 cm. in diameter at autopsy. These nodules on section were found to contain a caseoid mass, which was absent in only one case. The inguinal lymph nodes and lymphatics were enlarged, and in some cases there was induration of the surrounding fat tissue.

The gross lesions produced by intrapulmonary injection of lipins, extracted by both methods, also closely resembled each other. In all

cases consolidations were produced, and there were extremely dense and extensive fibrous pleural adhesions.

Histological study showed that hyperplasia was produced in the regional lymph nodes by injection of lipins of both methods. In addition, the Morse-method lipins produced hyperemia in some nodes. There was also a perfectly typical fibroblastic reaction, epithelioid in type, in some of the lymph nodes of guinea pigs receiving the Long-method lipins. An occasional giant cell was present here.

In the subcutaneous tissues, the lipins of both methods produced a purulent inflammation, sometimes phlegmonous in character, sometimes in the form of abscesses definitely walled off by either typical epithelioid tissue or granulation tissue of similar character, or both. Very few giant cells were present, but no caseation.

The chloroform-extract lipins produced on the pleura both a granulation tissue of epithelioid type and the usual fibroblastic reaction of similar type. In the lung itself, where the alveoli were not obscured by the proliferation of epithelioid cells or atelectasis, the alveolar walls were thickened, the epithelium desquamated, and the alveoli jammed with these cells and exudative materials. In some sections small areas closely resembling tubercles were seen, consisting of a central mass of cellular debris, staining with both hematoxylin and eosin, then an area containing only a few radially arranged epithelioid cells, the whole surrounded by the definite tissue of epithelioid type, which contained a few giant cells, although none were observed in the centres of these tubercle-like areas. Other areas present in the epithelioid tissue suggested small abscesses. There were also hemorrhagic areas, sometimes quite large, in the epithelioid tissue. The erosion through the bronchial wall, with lifting up of the mucosa, seen in previous cases, and accompanied by or due to a great proliferation of the lymphocytic elements of the lung, was also observed. A slight bronchitis with desquamation and fibrinous exudation was present. No areas suggesting caseation were seen in any specimens. The effects differed little with the chloroform extracts of the two methods.

4. *Injection of defatted bacilli:* In all cases a hard nodule succeeded the primary inflammatory reaction resulting from the injection. In only one case was caseoid pus visible in the gross specimen. In all animals the regional lymph nodes were somewhat enlarged, and in one the axillary nodes also. The lungs showed in two cases hard, grayish, nodular consolidations with grayish islands on section, but in two others no gross consolidations were evident.

Histologically, the regional lymph nodes were hyperplastic. Subcutaneous tissue taken from the inguinal region showed small areas of suppuration not noted macroscopically. The nodule described as containing caseoid pus was simply a larger abscess. Sometimes necrotic and hyaline muscle fibres appeared in the centres of these suppurating areas, surrounded on all sides by dead and disintegrated polymorphonuclear leucocytes, these in turn being surrounded by the fibroblastic reaction of epithelioid nature, in which case the area of polynuclear leucocytes merged gradually with the epithelioid tissue, or was surrounded by granulation tissue of similar type or by a tissue which was hard to distinguish from common granulation tissue. More giant cells than seen before in any section were present. The tissue was literally studded with them. Numerous epithelioid tubercles were scattered throughout, but none contained any giant cells.

On the pleura there was a fibroblastic reaction of epithelioid type, granulation tissue of the same nature and in some sections a dense hyaline scarring. In the lung itself both epithelioid granulation tissue and the simple epithelioid tissue were observed, the latter showing several epithelioid tubercles and numerous giant cells, and in some cases containing large areas of hemorrhage. No caseation was observed in any case.

INJECTION WITH LIPINS FROM GRASS BACILLI AND COLON BACILLI

The effects produced with these preparations were very similar to those obtained by injection of corresponding extracts of tubercle bacilli, and therefore need not be discussed in detail. In general, they produced a fibroblastic reaction of epithelioid type in the lungs and the subcutaneous tissues; but the ether and the chloroform extracts of the tubercle bacilli produced a similar reaction in the lymph nodes nearest the site of injection in some cases, which none of the nonpathogenic extracts or defatted bacilli were able to do.

In the lesions following the injection of nonpathogenic extracts and defatted bacilli, as well as in those following similar injections of tubercle bacillus extracts and defatted bacilli, there were numerous giant cells of the typical and foreign body type, these being a great deal more numerous in the tissues injected with the defatted tubercle and grass bacilli. These two emulsions, namely, the defatted tubercle and grass bacilli, produced very similar tissue reactions; but the defatted colon bacilli had a marked tendency to cause a scab formation and a superficial ulceration

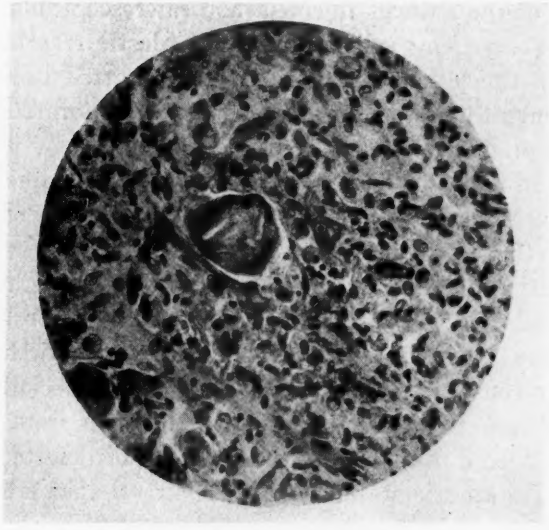

FIG. 1. LESION PRODUCED BY INJECTION INTO THE SUBCUTANEOUS TISSUES OF THE LIPINS EXTRACTED FROM COLON BACILLI. × 200

Note the similarity of the cellular reaction, including giant cell formation, to the tissue changes of tuberculosis. Similar lesions were produced by the lipins from tubercle bacilli and from grass bacilli, but not with the lipins from beef liver.

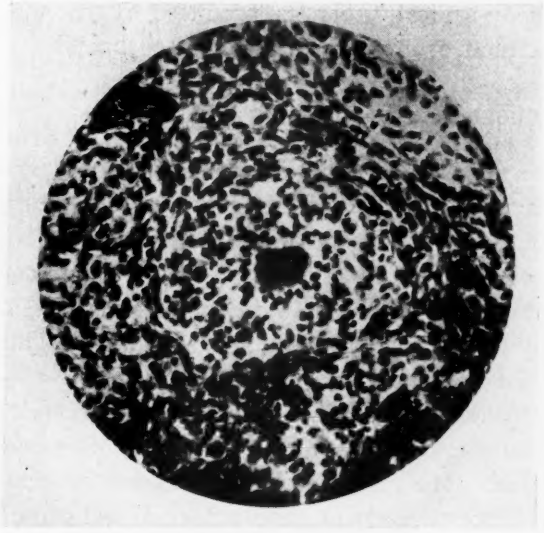

FIG. 2. LESIONS RESEMBLING ACTINOMYCOSIS PRODUCED BY SUBCUTANEOUS INJECTION OF DEFATTED GRASS BACILLI. × 200

in the groin of the animals injected, and microscopically these lesions did not show any giant cells and the fibroblastic reaction was not so marked as in the lesions produced by the defatted tubercle or grass bacilli, although there were many areas of abscess formation as in most of the lesions produced by the extracts of other bacilli.

The lipins of the colon bacillus produced quite the same effects as the lipins of the acid-fast bacilli, but less in degree and with a smaller number of giant cells (fig. 1).

The formation of purulent areas, filled with disintegrating polymorphonuclear cells and necrotic tissue, surrounded by a fibroblastic capsule and sometimes giving the appearance of actinomycotic lesions (fig. 2), was a rather constant finding in the lungs and especially in the subcutaneous tissues, following the injections of lipins from both tubercle and nonpathogenic bacilli and also injections of defatted bacilli. We cannot corroborate the statement of Auclair (7) that ether extracts of different bacteria produce the lesions that are characteristic of infection by each microörganism.

The chloroform extract of the tubercle bacilli and also of the nonpathogenic bacteria produced the most marked lesions of all the extracts, but only in the tissues injected with the chloroform extract of tubercle bacilli were definite epithelioid tubercles commonly produced. In few cases were these typical tubercles produced by the injection of nonpathogenic extracts or nonpathogenic defatted bacilli.

EFFECTS PRODUCED BY INJECTION OF FATTY MATERIALS FROM BEEF LIVER

These were much less than the effects produced by corresponding extracts from any of the three sorts of bacilli, as seen by the following description of the lesions observed in the injected animals.

1. Cold alcohol extract: Produced no lesions in lymph nodes, and a very small amount of fibrous reaction in the subcutaneous tissues near the site of injection in the right groin. The lungs showed a light desquamation of the bronchial epithelium and some atelectasis.

2. Hot alcohol extracts: Did not affect the lymph nodes, and the subcutaneous tissues at the site of injection in the groin showed only a slight fibrous reaction. The lungs showed an increase in small lymphocytes, some of which encroached upon the bronchial walls and broke through them in places. The bronchi showed a desquamating and exudative bronchitis.

3. Ether extract: Produced no visible lesions other than atelectasis of the lungs.

4. Chloroform extract: Caused some hyperplasia of the lymph nodes. Sections from the subcutaneous tissues and muscles in the region of the injection in the groin showed a proliferation of fibroblasts, giving rise to an indistinct fibrous capsule which surrounded what we took to be a chemical abscess, full of disintegrating polymorphonuclear cells. In some sections from this region the fat tissues were somewhat infiltrated by a fibroblastic tissue of epithelioid type, in which there was an occasional giant cell of foreign body type. Sections from the lungs showed a fibrous thickening of the pleura and many eosinophiles. There was a fibrosis about some of the bronchi and a slight amount of fibrous tissue of epithelioid type in parts of the lung tissue. There were also some areas of atelectasis, but none of the extensive epithelioid and fibroblastic proliferation produced by the bacillary lipins.

In conclusion, we can say that in no case did we produce true microscopic caseation, as Gaehlinger and Tilmant claim to have done with liver lipoids.

CONTROLS

In order to make sure that the results obtained were due to the fatty materials under consideration and not to the solvents injected with them, the following experiments were performed.

1: Injected a guinea pig in the lung and groin with 1 cc. of 0.25 per cent sodium stearate emulsion. This was much stronger than the concentration of sodium stearate used in emulsifying the lipins, in which from 3 to 5 cc. of a 1 per cent emulsion to 100 cc. of distilled water was employed, making the maximum concentration 0.05 per cent. The tissues at the site of injection were normal in every respect at the end of two weeks.

2: Injected in lung and groin with 1 cc. of 0.25 per cent sodium stearate emulsion, containing 0.5 per cent of chloroform. The lungs were normal. The skin over the area of injection in the groin was slightly thickened and edematous, but the reaction was a simple fibrosis and not even suggestive of the epithelioid type. No giant cells were present. The chloroform was injected because of the fact that the extensive lesions produced in the case of the chloroform lipins might have been accentuated by the small amount of chloroform which might possibly be present in the lipins extracted.

3: Injected in lung and groin with pure olive oil containing 0.5 per cent chloroform. This mixture was injected because of the possibility that the chloroform might produce more extensive lesions if injected in a menstruum which would delay absorption. There were no lesions, however.

4: Injected in lung and groin with 1 cc. of olive oil alone. In this case a slight amount of fibrosis in the subcutaneous tissue was produced. The lung was normal.

DISCUSSION

Morse and Stott found that the ether-soluble lipins of the tubercle bacillus were inert unless injected in amounts sufficient to cause pulmonary embolism. They, however, used white rats and rabbits as experimental animals, and it is possible that these animals are not susceptible to the action of the ether-soluble lipins, for our ether-soluble preparations were decidedly active in the guinea pig. A possible difference in the composition of the ether-soluble lipins, as extracted by Morse and as extracted by ourselves with his method, might account in some measure, though not entirely, for the differences in the results following injection of this lipin. Morse described his ether extract as a dark, viscous oil; ours was a dark red, waxy solid. The bacilli were allowed to stand in the cold ether for twenty-four hours by Morse, whereas we waited only twelve hours before pouring off the ether. This difference in the appearance of the extract may then be due to a more complete evaporation on our part, or to an actual difference in composition of the two extracts, it being conceivable that there may have been present in the bacilli some substance slowly soluble in ether, which by its presence in the final extract would change its physical properties and to some extent its pathogenic powers.

It is to be noted that, in our own experiments, using one species of animal throughout, and with the conditions for one animal being identical for all, our results varied; for instance, in only one case did any gross lesions result from intrapulmonary injection of alcohol-soluble lipins extracted by the Long method. In all experiments one must, of course, take into account the factor of individual resistance.

It was observed that lesions produced by the injection of lipins extracted by one method sometimes differed from the lesions produced by injection of the lipins extracted with the same solvent by means of the other method. The lesions following injection of ether-soluble tubercle

bacillus lipins, for example, extracted by the Morse method, were more severe than and differed from the lesions produced by the ether-soluble lipins extracted by the Long method. Further, it was noted that, in general, the epithelioid type of tissue seen in lesions induced by the Morse-method lipins was more vascular than that seen in the lesions caused by injection of Long-method lipins. There was also more hemorrhage associated with the lesions following injection of Morse-method lipins than was observed in lesions following injections of lipins extracted by the Long method.

It is hardly probable that the extracted lipins existed in the same chemical structure in the bacilli before extraction. Surely the heat and the succession of solvents would cause some molecular rearrangement, some change in melting point, or some change in solubility, so that the final product would differ greatly from the original material as contained in the bacillus. And, of course, different methods of extraction would cause different changes; the alcohol extract of tubercle bacilli, for instance, might contain different lipins or groups of lipins when the Morse method was used than they would contain when some other method was used. We must fall back upon some such explanation as this for the variations in the lesions produced. If the chemical structure of the tubercle bacillus were definitely known, and if it were possible to extract each separate constituent or group of constituents, and inject each separately, definite statements as to the effect of each substance injected could of course be made. But at present each observer uses a different method of extraction, which gives different products, and so results must vary. Until we know what we are injecting, statements as to the effects of injection of "alcohol-soluble lipins" or "ether-soluble lipins" of the tubercle bacillus must be regarded as being merely a shot in the dark as far as regards specificity of the materials injected.

Notwithstanding this lack of exact knowledge regarding the substances injected, there was a remarkable similarity in the tissue response to injections of lipins from the bacilli and the defatted bacilli themselves. With the exception of the presence of more giant cells and epithelioid tubercles in the lesions induced by injection of defatted bacilli, the lesions were alike. Indeed, one could not say with certainty from the microscopic picture alone, whether a given section was taken from an animal injected with tubercle bacillus lipins, defatted nonpathogenic bacilli, or living tubercle bacilli. Of course, the living tubercle bacilli

would in time produce caseation, which was absent in the lesions produced by the defatted bacilli and the lipins removed therefrom, but in the earlier stage the similarity of the lesions produced by these three agents is extremely impressive. One often sees uncaseated tuberculous lesions in the tissues of guinea pigs that do not differ at all from those produced by these lipins.

How can one explain this marked sameness in the tissue response to the injection of tubercle bacillus lipins, lipins from other bacilli, the defatted bacilli, and the intact (living or dead) bacilli? Shall one assume the presence of a toxic material which is present in all the extracts, as well as in the bacilli after the extraction? Or shall one assume that the fibroblastic reaction of epithelioid type, the tubercles, and the giant cells are merely the normal response of the body to an irritating foreign substance?

Since we obtained a tissue response with lipins from grass bacilli and colon bacilli, as well as with the defatted bacilli, exactly identical in all important respects to that obtained with tubercle bacillus lipins and fat-free tubercle bacilli, and since it is well known that subcutaneous injections of paraffin and many other substances frequently invoke a similar tissue response, it would seem that the latter assumption is the more likely explanation.

As far as we know, Auclair is the only investigator who has reported the production of caseation by means of injection of lipins of the tubercle bacillus. Since there exists in the serum of rabbits such quantities of antienzyme, antagonistic to the proteolytic enzyme liberated from the polynuclear leucocytes, that pus in these animals is hard and firm, suggesting in this respect caseation (8), and since Auclair used rabbits as well as guinea pigs in which this condition exists to a less degree, we must conclude that Auclair's "caseation" was simply solid pus. Fur ther, his description of the microscopic appearance of his caseation is far from convincing.

It would seem, then, that the body's response to the presence of the tubercle bacillus is not a specific defense built up by the invaded body, but merely a type defense,—a reaction invoked by many and varied substances, and in no way peculiar to tuberculous lesions. Apparently, the caseation seen in actual infection with the tubercle bacillus is due to the diffusion of some toxic product of bacillary metabolism, and is seen only when the bacilli are alive and the inflammation progressing (9).

SUMMARY

1. Tubercle bacillus lipins and the defatted bacilli possess the power of exciting a local fibroblastic reaction of epithelioid type, with tubercles, giant cells, and abscesses, when injected into the lungs and subcutaneous tissues.

2. Hyperplasia of the regional lymph nodes was constant. Fibroblastic proliferation of epithelioid type was produced in the regional lymph nodes only by the ether and chloroform-soluble lipins.

3. With the ether-soluble lipins extracted from tubercle bacilli by two methods, and the chloroform-soluble lipins extracted by the Long method, sufficient proliferation of the lymphocytic elements of the lung was produced to erode the bronchial walls.

4. Definite epithelioid tubercles were produced only with the chloroform-soluble lipins and with the defatted bacilli. Far more definite tubercles were present in lesions induced with the defatted bacilli than in lesions induced with the chloroform-soluble lipins.

5. Giant cells were present in the lesions produced by injections of all the bacterial lipins tested, but were far more numerous in lesions produced by means of the defatted acid-fast bacilli.

6. In general, the lipins extracted by the Morse method produced a more vascular epithelioid tissue, with more associated hemorrhage, than was produced with lipins obtained by means of the Long method.

7. Lipins extracted from grass bacilli and colon bacilli by similar methods produce effects entirely similar to those produced by the tubercle bacillus, except that with the latter there was more involvement of the regional lymph nodes. Evidently, then, the characteristic histological features of tubercle cannot be considered to be specific effects of the lipins of the tubercle bacillus, but merely a foreign body reaction, perhaps slightly modified by the water-soluble products of tubercle bacilli.

8. Defatted tubercle bacilli and grass bacilli may produce typical epithelioid cell lesions with many giant cells. Lesions identical with those of actinomycosis may also result. Defatted colon bacilli produce similar lesions but with no giant cells.

9. No areas of true caseation were observed in any section. Probably the supposed production of caseation with lipins from tubercle bacilli and beef liver reported by others depends on the solid character of the pus produced in rabbits and guinea pigs, which macroscopically closely resembles caseous material.

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STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS

XXVI. FAT-SPLITTING FERMENTS IN LYMPHOCYTES

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The prominence of the lymphocytes in the body's reaction to infection by the tubercle bacillus has naturally led to inquiries as to the part they may play in this defense. In 1909 Bergel (1) reported experiments which were interpreted by him as establishing the presence in lymphocytes of active fat-splitting ferments, to which he attributed their supposed capacity to attack the tubercle bacillus.

Tuberculous exudates and spleen and lymph node juice were placed on plates and allowed to stand in an incubator at 52°C. for twenty-four hours. The plates were made from stearin, palmatin, and white and yellow wax, the latter with a melting point of 63° to 64°C. It was not specifically stated whether or not the yellow and white wax was beeswax, but presumably it was. The plates showed no digestion or depression in the stearin or palmatin, and various lymphocytic materials simply dried up. On the yellow wax plates, especially, characteristic depressions with crater-like hollows appeared and these were surrounded by a well defined ridge or wall, so that the wax had the appearance of having been digested or eaten out by the lymphocytes.

Control tests were made, using, in place of the lymph materials, physiological salt solution, tuberculin, fibrin and trypsin, all of which dried up and gave no depression in the wax.

The production of these hollows with the raised margins was interpreted as indicating that the lymphocytes had digested the wax, and, on the basis of these observations and this conclusion, the suggestion was made that the importance of lymphocytes in tuberculosis lies in their capacity to attack the waxy constituents which form so large a part of the substance of the bacillus. This hypothesis has been conspicuous in the tuberculosis literature since Bergel's paper, although it has not been submitted to very extensive reinvestigation.

Caro (2) performed some experiments with monobutyrin and tributyrin, which he found to be split by lymphocytes, but apparently he did not repeat Bergel's identical experiments. From his own observations and the reports in the literature he drew the conclusion that it has not yet been positively shown that lymphocytes contain fat-splitting ferments.

Nees (3) carried out a large number of experiments similar to Bergel's. Beeswax alone was used in part of the experiments and mixtures of beeswax and various other substances, such as paraffin, hog fat, etc., together with an indicator, methyl red, were used in the last part of the work. The cellular exudates from a wide variety of disease conditions were placed on the different kinds of plates. The results obtained by Nees were not constant. Sometimes some plates in the same series with the same kind of cell material gave results indicating a splitting of the fat, while other plates in the same series showed no change. There was no explanation offered for these discrepancies or differences. Nees's conclusions were (1) lymphocytes as well as leucocytes split fat, (2) the ability to split fat is a general cell property, and (3) caution must be used in judging results, especially in applying them to tuberculosis as Bergel had done. Since then Bergel has published other articles (4), which he believes also support the hypothesis that lymphocytes are important in tuberculosis because of their ability to attack fats and waxes. Recently Aschoff and Kamiya (7) have taken exception to Bergel's claims that he has shown that lymphocytes possess any characteristically high capacity for the disintegration of lipoids, but they did not reinvestigate his experiments with the wax plates.

As we have on hand in our laboratory considerable quantities of different lipins extracted from tubercle bacilli, it was suggested that it would be desirable to determine whether the enzymes of lymphocytes which split other fats and waxes are capable of attacking the lipins of the tubercle bacillus. This would serve to throw light on the reputed significance of lymphocytes in tuberculosis as destroyers of tubercle bacilli through their ability to attack the latter's waxy material. I therefore undertook, first, to repeat Bergel's experiments, in order to acquire familiarity with his technique and results, before applying the same technique to the lipins obtained from tubercle bacilli.

EXPERIMENTAL METHODS

In my preliminary experiments the tissues containing lymphocytes were macerated in fine sand and about 10 cc. of tap water were added

for each 2 gm. of tissue. Five cubic centimetres of the resulting emulsion was then boiled on a water bath for one-half hour in order to destroy the enzymes. This portion was used for control experiments. To each 5 cc. portion was added 0.5 cc. of toluene to prevent putrefaction, and small drops of each emulsion were then placed on plates of the different materials to be tested.

In the second set of experiments the different lymphoid tissues to be tested were placed directly on the wax plates without making an emulsion with water or adding toluene.

Finally, the lymph nodes were scraped with a knife, in order to obtain cellular material, and this was placed on the wax plates, for it was thought that by so doing the "sinking in" of the solid tissue could be eliminated and the exact effect of the lymphocytes could be observed.

The plates were glass containers, about 3 cm. in diameter and 1 cm. deep, with straight sides and flat bottoms. The different fats and waxes were melted and poured into these plates and allowed to cool to room temperature before the tested materials were placed on the smooth top surface of the wax. In some of the experiments methyl red was added directly to the melted fat or wax before pouring into the plates, according to the method used by Nees. After placing the material evenly on the plates, they were carefully covered with glass lids and the edges sealed with melted beeswax. The plates were then placed in a porcelain dish containing a little water and the dish in turn was covered with a lid and its edges carefully sealed with melted beeswax. It was found necessary to do this to prevent the lymph material from drying, especially when incubated at 52°C.

RESULTS OF EXPERIMENTS

1: In the first set of experiments the materials used were from guinea pigs. They comprised (1) normal lymph nodes, (2) normal spleen, (3) caseous, tuberculous spleen, and (4) caseous, tuberculous peritoneal lymph nodes. The material was made into an emulsion and toluene was added. Controls were run with boiled material. Beeswax and lanolin were used, and three plates made in each case, two for the normal tissue and one for the boiled, and each incubated at 37°C. for twenty-four hours.

No visible change resulted in any of the beeswax plates. The emulsion sank into the lanolin in every case, whether of boiled or fresh tissue, but there was no visible digestion.

2: A second set of experiments was carried out as above, with the exception that methyl red was added to the plates prepared from lanolin, stearin and beeswax. The tissue used here was from normal guinea pig pancreas and lymph nodes.

The plates showed no digestion in any case and no color reaction on any, except on the stearin plates with the unboiled pancreas emulsion, which showed a bluish ring about the drops of emulsion.

3: In place of the emulsified tissue material, the tissue was placed in small pieces directly on beeswax, lanolin, and half lanolin, half beeswax plates, to which methyl red had been added, and these plates were then incubated forty-eight hours at 52°C.

The plates showed no color change, but there was a slight depression in all plates, with the boiled as well as with the fresh material.

4: Plates were also made from the wax or fat from tubercle bacilli prepared according to Long's method (5), chloroform soluble fraction, and also from beeswax.

The material used here was normal guinea pig (1) pancreas, (2) spleen, (3) lymph nodes, and also tuberculous human lymph nodes. The plates in each case were kept at 37°C. for twenty-four hours and a second set at 52°C. for forty-eight hours. They showed a slight depression in the beeswax and considerable depression in the tubercle wax, even with the boiled lymph material. The depressions were deeper in the plates incubated at 52°C.

5: Plates were made as in *3* and *4*, but the cellular material was secured by scraping the lymph nodes with a knife. Inert substances, such as beeswax and small pieces of wood, salt solution and water, were used for controls, and the plates were incubated forty-eight hours at 52°C. There was a slight depression with each material, whether fresh cells or inert substances.

DISCUSSION OF RESULTS

Early in the work it was noticed, especially with the lanolin and later with the wax of the tubercle bacilli, that the lymphatic material had sunk down into the plates, and at first it was thought that this was due to digestion of the fat by the enzymes present in the lymphocytes. But when boiled tissues, and later even inert material like beeswax, water and salt solution, also sank in when incubated, it became evident that there was no digestion at all, but rather the depressions in the wax

were really due to the "sinking in" of the materials used in the incubator, especially since this was more noticeable at 52°C. No difference whatever could be discerned in respect to the character of the depression produced by fresh tissues and inert controls. Indeed, when some of the plates of beeswax and tubercle wax were compared with the description of results and a cut given by Bergel (1), it is easy to see how this "sinking in" of lymph material could be mistaken for a digestion. The raised margin or rim about the depression in the wax is probably compensatory to the sinking in of the surface, *and is of itself evidence that the depression is simply mechanical rather than the result of digestion.*

The results obtained by using the pancreas as a source of lipase are especially important. It was found that the only evidence of fat splitting or fat digestion, even with this lipase-rich organ, was with stearin. However, there was no characteristic "walled about depression" here, such as Bergel describes, only a slight change to acid indicating a breaking down of the fat molecule. With stearin, lymph nodes gave no color reaction. Indeed, in no other experiments was there seen any change in the indicator which would indicate digestion.

That negative results were obtained is probably due to the fact that the wax molecule, in the case of each of the waxes used, was too stable to be attacked by lipase even if this enzyme were abundant in the lymphocytes. This will be made evident from a consideration of the chemistry and hydrolysis of beeswax.

According to Lewkowitsch (6), in order to hydrolyze beeswax it requires one hour of boiling with strong alcoholic potash made with at least 96 per cent alcohol. The difficulty with hydrolyzing the solid waxes is probably due to the fact that the resulting sodium soaps are less readily soluble than those obtained from hydrolyzed fats, and hydrolysis does not occur in steps or stages as in the case of simple fats. In our experiments even fresh pancreas produced no evident digestion of beeswax.

From these considerations it is seen that beeswax offers a considerable resistance to measures that are commonly efficacious in hydrolyzing ordinary fats and, therefore, it would appear unlikely that beeswax could be digested by ordinary tissue enzymes.

Beeswax has many properties similar to the fatty substances which form a considerable part of the bodies of tubercle bacilli. These fatty substances not only exhibit a very low iodine value but have a strong resistance to measures that commonly saponify ordinary fats.

CONCLUSIONS

1. There was no demonstrable digestion or fat splitting of the wax of tubercle bacilli, beeswax or lanolin by material containing lymphocytes when tested by the plate method of Bergel. This is thought to be due to the fact that the waxes are so stable that they cannot be hydrolyzed or digested by ordinary tissue enzymes. No digestion was observed even with fresh pancreas, which is known to contain strong lipase.

2. The depressions that were observed in the wax plates were apparently due entirely to sinking in of the tested material, while the wax was softened in the incubator, and not due to a digestion of the wax, since depressions similar in character were produced by boiled tissues and cells, as well as by entirely inert substances.

3. There was no change in reaction (as shown by the methyl red indicator) produced by any of the materials containing lymphocytes on tubercle bacillus wax, stearin, beeswax or lanolin, either in twenty-four hours at 37°C. or in forty-eight hours at 52°C., as would be expected if there was any digestion or hydrolysis of the wax.

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DEMINERALIZATION IN TUBERCULOSIS

DISTRIBUTION OF CALCIUM IN THE TUBERCULOUS GUINEA PIG

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Demineralization is the loss of inorganic material in various tissues in consequence of infection. In the infected animal, it is thought that the concentration of such material is high in normal and reduced in diseased tissues.

Robin (1), the chief advocate of this theory says that "analysis of the lung of a normal man compared with that of healthy and diseased parts of a tuberculous lung shows that tuberculous parts are demineralized in high degree while, on the contrary, healthy parts are overmineralized to an equivalent extent," and that "this overmineralization is therefore to be considered as a defensive reaction."

Von Noorden (2) reports loss of calcium in tuberculous individuals: "in phthisis calcium is excreted by the urine in markedly greater amounts than in normal people."

Robin bases his theory on the analyses of tuberculous and normal tissue, while Von Noorden argues on the basis of metabolic experiments.

It occurred to the investigator that it would be desirable to determine the concentration of the various cations in tuberculous animals. Von Noorden's method was not appropriate; for there may be a retention of certain elements, in the metabolic sense of the word; nevertheless, normal tissue may lose these elements while they may be stored in diseased tissue. Furthermore, this method would not explain the following condition: The protein molecule of the disintegrating tissue may be split and its metal used by the normal tissue. A blood analysis for the respective elements would not be of much value; for the blood may be rich in the matter in question, such a high concentration being due to the demineralization of the diseased tissue.

Robin's analyses were made for the total and inorganic ash; it is, however, important to know the actual amount of the elements in the tissues, in the discussion of such a problem.

[1] Fellow of the Trudeau Foundation.

In the following series, the secretory organs were chosen as tissue and calcium as the element. The secretory organs were taken because they are easily affected by experimental tuberculosis; calcium on account of its supposed importance for the issue of this disease. Full grown guinea pigs were used as subjects, some of them having been used as infection controls in other experiments.

The organs were dried immediately, or within six hours after the death of the animal. They were freed from fat and connective tissue, cut into small bits, and air-dried by means of an electric fan; then ground in an agate mortar and dried over sulphuric acid to constant weight. One-half to 1 gm. of dried tissue powder was used for analysis. This was incinerated in a platinum crucible which was protected by a Gooch crucible; in this way a very uniform heating was made possible. The white ash was dissolved in 1 to 5 cc. 1/N hydrochloric acid, and distilled water was added to fill the crucible, which was then covered and heated for two hours on the water bath. The liquid was evaporated so that only 4 to 5 cc. remained; this was transferred to a 15 cc. centrifuge tube; one drop of 2/N nitric acid was added and the tube heated for ten minutes. The iron was precipitated as phosphate with ammonium acetate. After centrifugation the supernatant fluid was transferred to a 50 cc. centrifuge tube and the calcium precipitated at 100° as oxalate in a weak acetic acid solution. After twenty-four hours standing in the icebox, the tube was centrifugated, the supernatant portion discarded and the precipitate resuspended in 40 cc. of a 1 per cent ammonium hydroxide solution. After washing, the calcium oxalate was dissolved in 4 to 6 cc. 1/N sulphuric acid and titrated at room temperature with 0.01/N potassium permanganate, the latter being standardized against iron.

Animals were infected with small doses of the Saranac Laboratory strains, R1 and H60, the former being of attenuated, the latter of moderate virulence. Suspensions were prepared of such concentration that they contained from 5 to 10 single bacilli to an oil-immersion field. Calcium was administered by subcutaneous injections of calcium chloride, and as inhalation or subcutaneous injections of marble dust suspension. In no tissue analyzed was there macroscopic evidence of calcification.

The analytic results are tabulated as follows, per cent being expressed in grams of calcium per 100 grams of dried tissue.

A guinea pig (401–5B) was infected with 0.5 cc. H60 suspension, March 9, 1922. In the course of another experiment it was exposed from this date to its death to marble dust inhalations. Died May 26th. *Autopsy:* generalized chronic tuberculosis, spleen eight times normal size, bloody fluid in peritoneum, all other tissues very pale.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.1526	0.06175	0.5784	0.06542
0.1952	0.06095	0.4613	0.05771

Animal 401–3B was treated the same as 401–5B. Died June 1st. *Autopsy:* generalized chronic tuberculosis.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.1486	0.08445	0.06927	0.04024
0.1475	0.09968	0.06559	0.04451

Animal 401–12B was a control animal, infected like 401–5B but not exposed to dust. Died June 1st. *Autopsy:* generalized chronic tuberculosis. Lungs, liver, spleen, and kidneys very large but no gross disease.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.05157	0.0797	0.3419	0.06131
0.04955	0.06842	0.3442	0.05666

Animal 401–13B was treated like 401–12B. Died May 17th. *Autopsy:* marked generalized tuberculosis; fibrocaseous involvement of inguinal lymph nodes, omentum, spleen, liver (enlarged) and lungs; tracheobronchial lymph nodes much enlarged.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.05945	0.10015	0.08112	0.05891
0.06269	0.0998	0.07091	0.05813

Animal 401–14B was treated like 401–12B. Died May 22nd. *Autopsy:* the same as 401–13B.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.09512	0.1344	0.5952	0.05701
0.1088	0.1031	0.5739	0.05392

Animal 400–2. This animal was infected, July 18, 1921, by inhalation with an atomizer, receiving five puffs of an R1 suspension. Died April 26, 1922. *Autopsy:* extensive healing tuberculosis in lungs and tracheobronchial lymph nodes.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.03302	0.04047	0.03624	0.04933
0.03305	0.04276	0.0364	0.07827

Animal 401–16B, a normal guinea pig, received two doses of calcium chloride, each containing 0.75 gm. Ca. Died March 17th. *Autopsy:* ulcer in deep tissue at sight of inoculation; no other lesion.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.04354	0.05241	0.06693	0.039
0.04025	0.04797	0.0627	0.04157

Animal M–401–12A. Was infected December 17, 1921, with 0.4 cc. R1 subcutaneously. Following this, at irregular intervals, it received injections of heavy marble dust suspension in the subcutaneous tissues of the same groin. Killed March 14, 1922. *Autopsy:* superficial inguinal lymph nodes moderately enlarged and caseated. No evidence of calcification.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.06188	0.04208	0.6188	0.04196
0.06364	0.03944	0.7039	0.04311

Animal 420–2, infected with 0.5 cc. R1, subcutaneously, on April 27th, and exposed to marble dust like 401–5B. Died June 2nd. *Autopsy:* Diaphragmatic abscess, no trace of infection.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.0506	0.03128	0.09361	0.0436
0.04945	0.03475	0.09766	0.05023

Besides the above infected animals the tissues of three full-grown normal controls were analyzed; the results were as follows:

Animal C1. Killed on March 26th.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.03213	0.03998	0.03227	0.03743
0.03193	0.04958	0.03773	0.04387

Animal C2. Killed on August 5th.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.03425	0.05642	0.03262	0.03933
0.03927	0.05609	0.02945	0.04224

Animal C3. Killed on August 19th.

LUNGS	LIVER	SPLEEN	KIDNEYS
per cent	*per cent*	*per cent*	*per cent*
0.02983	0.05381	0.04186	0.044379
0.03263	0.05405	0.04098	0.04257

From this account we find the calcium concentration in the tissues analyzed of normal guinea pigs quite constant.

In discussing the ratio of the calcium distribution in various organs of infected guinea pigs, we may classify them into two groups. The animals infected with the moderately virulent H60 generally show the highest concentration, those exposed to marble dust (401–5B and 3B) being especially large. On the other hand the tissues of animals exposed to dust inhalations plus infection with R1, infection with which strain is uniformly followed by spontaneous healing in the organs of the guinea pig, show a slight increase in calcium content, but not more than may be accounted for by individual variation.

These findings are in accordance with the view taken by Bauer (3), Von Kossa (4), Lichtwitz (5), and Wells (6), that areas of pathological calcification are areas where fatty degeneration takes place; therefore we see in the organs which are not attacked by the disease, such as the kidneys in the tuberculous guinea pigs, only small variations in the

calcium content. It must, however, be pointed out that none of the tissues in question showed macroscopic calcification. The high calcium concentration in the spleens and livers of the H60 animals may be explained as a calcium metastasis in Virchow's sense.

An appreciable loss of calcium due to progressive tuberculosis could not be proved in any case. The spontaneously healed tuberculous lesions produced by infection with the low virulent R1 strain, which should, in the light of the theory of demineralization, yield a low calcium content, showed, on the contrary, quite normal values.

There may, nevertheless, be a diminution of other inorganic matter which should be taken into consideration before sweeping deductions can be drawn.

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THE DISH TOWEL AS A SOURCE OF TUBERCULOUS INFECTION

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The demonstration of ingestion as an important source of tuberculous infection has led to a search of all avenues by which the infection may be acquired in this way. Extensive investigations have been made of room dust contaminated with sputum. The possibility of infection of many points of the digestive tract through the ingestion of tubercle bacilli is well known.

The results of Price, Brown and their co-workers, in demonstrating the ease with which tubercle bacilli could be obtained from dishes used by the "open" consumptive has stimulated active interest in this avenue as an important mode of possible family infection. Furthermore, occasionally it has been demonstrated that even careful cleansing of such table utensils does not always remove this danger. When one considers the number of bacilli expectorated by the tuberculous and the lack of care exercised in the cleansing of eating utensils day after day the wonder is that repeated self-infection is not a constant sequel of the disease.

The present problem has involved the study of the dish towel as a source of tuberculous infection. In a previous investigation by one of us, on the infectivity of eating utensils, material was secured from twenty-five homes. In this work the same group furnished the material used. As tubercle bacilli have been repeatedly demonstrated on eating utensils and in the wash-water, the chance of repeated infection of tableware from the dish towel would seem likely to be a common occurrence. The homes from which the specimens were secured were those of an average tuberculous family. In each home there was a case of active phthisis from which the sputum was repeatedly positive. The care taken by the patient to control his infection was of the best. The dish towel, after its use, was generally rinsed out in hot soapy water and hung up to dry. The specimens secured for study had been in

service for some time. They were of coarse material, such as huckaback or something similar, and of some thickness. Each towel was placed in a sterile container and brought to the laboratory. They were often foul-smelling and very greasy. The centre of the cloth was cut out, divided into small pieces with sterile instruments, and placed in a flask of salt solution. Prolonged vigorous shaking of the flask and its contents in many instances produced a very turbid solution. The soaking bits of towel were then placed in the cold room for twenty-four hours, after which a second thorough shaking was given the material. The bits of cloth were then removed and squeezed and cultures were planted from the towel washings. In many instances the cultures showed numerous colonies, so that the dish-cloth water had to be returned to the cold chamber for frequent freezing and thawing, to kill out extraneous microörganisms before guinea pigs could be safely inoculated. It should be noted that the majority of the dish towels received had for many weeks or months been saturated with a solution of strong alkali soap or washing powder. Naturally, therefore, the wash-water of these towels contained a moderate amount of alkali, to which any viable tubercle bacilli which were present were subjected, during the period that the wash-water was in the cold chamber.

In each instance two full grown animals were used and both the abdominal wall and the peritoneal cavity were inoculated. About 2 cc. of the centrifugated washings of the towels were injected into each guinea pig. No reaction to the material followed. It was intended to keep the animals under observation for from six to eight weeks, but in some instances the guinea pig died from pneumonia before this period was up. Among the 50 guinea pigs so inoculated, all were found to be negative at autopsy. Any animals showing the slightest enlargement of any of the lymph nodes had these removed, crushed and stained for tubercle bacilli. In no instance were they positive.

In order to control these negative results, three pieces of clean gauze were plentifully saturated with a viable culture of tubercle bacilli. The bacilli were sprayed upon the gauze for three successive days, and on the fourth day the cloth was thoroughly shaken in heavy soapsuds. The soapsuds were drained off and the cloth well rinsed in salt solution. Following this primary washing, the spraying was again repeated for three days, the cloth thoroughly washed and the process repeated for the third time. Following the third washing, the gauze was cut into small pieces and allowed to stand in salt solution in the cold room over night.

It was then vigorously shaken for about twenty minutes and the wash-water thoroughly centrifugated. The sediment was inoculated into the peritoneal cavity of a guinea pig. This process was carried out with three pieces of gauze, the only change in the method being that different soaps or soap powders were used, such as are commonly found for washing purposes in the homes of the tuberculous. At the end of two months the three animals inoculated from the wash gauze were killed and all three were found negative for tuberculosis.

SUMMARY

1. In a series of 25 instances where guinea pigs were inoculated from the washings of dish towels used by tuberculous patients no positive results were obtained.

2. In three control experiments, where gauze was thoroughly impregnated with viable tubercle bacilli and thoroughly washed, no positive results were obtained.

DISCUSSION

In view of the fact that it has been demonstrated that the dish-water from the eating utensils used by the tuberculous frequently harbors virulent tubercle bacilli, it will seem rather unusual that the dish towel frequently employed in such water should not harbor them in sufficient numbers to allow of guinea pig infection, when the sediment from them was injected into the peritoneal cavity. Freezing and thawing of the material may have given some negative results, but negative controls were not subjected to this process. The most reasonable explanation of these negative results would seem to be that the strong alkali soap or soap powder used over a period of weeks in the home must very deleteriously affect any viable tubercle bacilli that may be caught in the meshes of the dish towel and either kill them or so impair their vitality as to make them unable to produce infection in small numbers.

APICAL TUBERCULOSIS

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Recent literature on pulmonary tuberculosis contains comparatively little discussion of the question of the localization of tuberculous infection in the lung apex. In general the question has not been regarded as settled.

Freund (1), as early as 1858, advanced the theory that a shortening and ossification of the first costal cartilage caused the first rib to press unduly upon the lung apex and that this pressure predisposed the apex to tuberculosis.

Birch-Hirschfeld (2) called attention to the peculiarly shaped apical bronchi which have the appearance of having been stunted, and expressed the opinion that the shape of the apical bronchi was the factor which rendered the apices of the lungs susceptible to tuberculosis. Schmorl (3) added that the peculiarity of the bronchi and the results thereof, in causing apical tuberculosis, were due to the furrow made across the apex by the shortened, firmly fixed first rib.

Hart (4) made several observations, which he thought might have an influence in causing the lung apex to be a favored site for tuberculosis, as follows: the sternal end of the first rib is lower than the spinal end, and this causes a compression of the apex, which would not exist were the first rib horizontal; the changes in the first rib cartilage may be asymmetrical as regards the two sides of the chest and one apex may be compressed more than the other; in the pathological state of the upper aperture of the thorax, the transverse diameter is shortened and, what is of paramount importance, the paravertebral areas, in which the apices lie, may be much contracted; and the shape of the vertebrae of the region may be such that they also impinge upon the apices. Hart concluded that the malformed slanting first rib girdle compresses and injures the lung tissue of the apices, interferes with the ventilation thereof, and embarrasses the blood and lymph flow, and hence subjects the apices unduly to tuberculosis.

Bacmeister (5), Rothschild (6), Hart and Harrass (7), Martius (8), and others accept the view that apical tuberculosis is caused indirectly by the pathological upper aperture of the thorax.

Tendeloo (9), Keith (10), and others have explained apical tuberculosis by the lessened motion and a consequent retarded blood and lymph flow of the apices as compared with other portions of the lungs. Keith emphasizes also the lack of expansion as of paramount importance. Tendeloo's law that every portion of the lung is dependent functionally upon the overlying portion of the thorax, in conjunction with the fixed first rib aperture, explains why there is relatively little movement of the apices in certain cases.

Stiller (11) attaches some importance to the first rib anomalies in the selective action of tuberculosis for the apices, but he thinks that this is only one of many factors.

Schiele (12) stresses the downward tilting of the first ribs as the cause of the interference of the function of the apices,—the greater the tilting the greater the interference. Schiele and Révèsz (13) regard the early ossification of the first cartilages as a result and not as a cause of the tuberculosis.

Pottenger (14) says that the compression of the apices, by the shortened first costal cartilage and the other malformations of the upper thoracic aperture, is only a part of the general compression of the upper portion of the lungs in the adult, and that this explanation bases the infection of the apices upon anomalies instead of upon "natural anatomic and physiological factors." He explains that in the child at birth the first ribs are horizontal, the antero-posterior diameter is relatively great, and the chest is rotund. The anterior portion of the chest begins to sink and the upper portion flattens, as the child assumes the erect position, and by the sixth year the top of the sternum has fallen from the level of the first thoracic vertebra, or even of the seventh cervical, to the level of the disc between the second and third thoracic vertebrae. The lung of the child grows in size far out of proportion to the growth of the chest; and this is notably emphasized in the upper thorax, which is really flattening and narrowing in the growing child; therefore, the apices of the lungs undergo increasing compression as the child grows to adulthood. The squeezing together of the lung tissue of the apices, which has developed, causes a narrowing of the lymph spaces and air cells and a decreasing of the respiratory movement, with its consequent retardation of the blood and lymph flow. Pottenger thinks that the

decreased amount of lymph and its sluggish flow in the apices may be factors in reducing the resistance of the apices to the tubercle bacilli. He holds that his explanation accounts for the infrequency of tuberculous infection in the apices in children, as well as for the predilection of the apices of the adult for tuberculosis.

Fishberg (15) says that some have suggested that the determining factor is the blood content in the apices, and that it has also been found that in the upper parts of the lungs the blood and lymph currents are slower than in other parts, and thus embolic deposits of bacilli are favored, no matter by which channel they have entered. The uppermost three ribs, he continues, show smaller respiratory excursions than the lower ribs, and the result is a slower air current in the upper part of the lungs; but these and many other hypotheses, he adds, have failed to adequately explain the apical localization of phthisis.

Van Zwaluwenburg and Grabfield (16) have observed a thickening of the apical pleura in early tuberculosis; and it is their contention that tubercle bacilli from tuberculous infection of the lymphoid tissue of Waldeyer's ring of the pharynx passes to the "cervical lymphatics, to the apical pleura, and thence into the lung;" in this way they account for the frequency of apical tuberculosis.

Pfeiffer (17), Grober (18), Klebs (19), Beckmann (20), and Wassermann (21) also held that the tubercle bacilli reached the lung apices directly from the supraclavicular lymph nodes, which had been infected from tuberculous areas in the pharynx.

Grober (22) injected india ink into the pharyngeal tissues of animals and found that the cervical lymphatics deposited it upon the apical pleura and that it was then passed through to the parenchyma of the lungs.

Beitzke (23) and Most (24) denied that there could be any lymphatic channel whereby bacteria could reach the lung apex from the cervical nodes.

Shaw (25) found that tubercle bacilli introduced into the body of a rabbit which had one collapsed lung invariably located in that lung; he says that "the collapse of the lung changes the resistance of the animal at the point of collapse or acts in some mechanical way," and asks, "Would this not be true of atelectactic areas in the lungs?" He adds that "the action of the muscles on the upper ribs of the thorax, as shown by Hoover, probably plays a rôle in the localization of tuberculosis in the apex of the lung." Hoover (26) says, "The function of the scaleni

muscles is solely to form a point of fixation in the first rib from which the second, third, and fourth ribs are elevated."

Phillips (27) advances the view that the predilection of the apices for tuberculosis is due to the orthograde posture which produces an anemia of the apices, gravity causing the blood to circulate more liberally in the lower portions of the lung than in the apices. He states that the capacity of the lungs for blood is greater than the amount of blood ordinarily passing through the lungs, and that thus it is easy to see why the apices might become anemic from the orthograde posture.

Aufrecht (28), Gördeler (29), Grawitz (30), Behring (31), Thompson (32), Pottenger (33), Hildebrandt (34), Buttersack (35), v. Weismeyer (36), and Harbitz (37) believed that tubercle bacilli could pass from the cervical lymphatics to the bronchial lymph nodes, whence they were transferred to the lung tissue by the blood.

Most (38) has demonstrated, in man, that there is a connection between the deep cervical lymphatics and the tracheobronchial nodes.

Both Gray's (39) and Gerrish's (40) textbooks of anatomy say that the deep cervical lymphatics communicate with the mediastinal nodes and these in turn with the tracheobronchial nodes. The bronchial nodes gather the lymph from the lungs and Miller (41) says no lymph leaves the lungs without passing through the bronchial nodes.

Beitzke (23), however, believed that there is no connection between the cervical lymphatics and the bronchial nodes; the weight of the evidence speaks positively for anastomoses from mediastinal nodes to bronchial.

Krause (42), in experiments on guinea pigs, has found that tubercle bacilli entering the body in sufficient numbers, irrespective of the site of entrance, invariably reach the tracheobronchial lymph nodes, if not arrested at some more peripheral point. The bacilli may or may not produce lesions in the lungs. If the bacilli are injected subcutaneously, they slowly pass centralward to the nearest node and thence to the next and the next, until they are discharged into the blood. As the bacilli-laden blood passes through the lungs, some bacilli are carried out of the finer blood vessels and enter the lymphatics, to be passed on to the tracheobronchial nodes. He says, "The only way that so many bacilli could have come to the tracheobronchial nodes is lymphatically from the lungs; and, since they must have entered the lungs by the blood, to get into the intrapulmonary lymphatics they must have first been temporarily fixed in the blood vessels and carried, through the walls of the

latter, out into the tissue spaces and lymphatics." Krause argues that tubercle bacilli are so frequent in lymph nodes because they accumulate in relatively great numbers in the nodes, by virtue of being carried into lymphatics. Speaking of tuberculosis of a lymph node, he says that tuberculosis of a lymph node tends to become arrested unless there is a more or less constant feeding of bacilli to the node from a peripheral focus. To quote from him, "We would at once wonder whether in general the clinical outbreak of tuberculous lymphadenitis in human beings were not largely determined by repeated infections from more peripheral and concealed foci, as, for instance, in tonsils or in adenoid growths in the nasopharynx."

Rogers (43) has demonstrated that guinea pigs inhaling a spray of tuberculous sputum, even for no longer than five minutes, develop tuberculosis; tubercles were produced in the lungs with a concomitant involvement of the cervical, mediastinal, and bronchial lymph nodes.

Shingu (44) found that dust inhaled by the adult was deposited in the lung tissue, especially of the apices, whereas in children the inhaled dust located in the bronchial nodes.

Pottenger (14) makes the significant suggestion that possibly "the reason for the marked deposit of soot in the lung tissue in the adult is because, through protracted exposure, more and more particles have become deposited in the lymph spaces and that these retard the progress of others taken in later."

For bacteria to grow in a definite location in the body certain known factors may be operative. Highly virulent microörganisms may be implanted upon a normal tissue and, by virtue of their ability to grow rapidly, they may gain a foothold; or perhaps the toxin of the bacteria is so destructive to the tissues of the part that a favorable culture bed for the bacteria is produced.

A mechanical injury to the tissues produces a site upon which bacteria of even low virulence may grow. A pocket or crevice about the body may be so located as to pick up bacteria and organic waste, and thus, with the natural heat of the body, is afforded an ideal culture bed and incubator for the bacteria.

Previous disease may have affected an area so that it has a diminished blood or lymph supply, or both, and thus may be responsible for the selection of a site by an entirely different group of bacteria than that originally growing at that place. The endothelial lining of the capillaries might be so diseased as to favor the lodgment therein of infected emboli, or of bacteria floating free in the blood or lymph.

Bacteria have their optimum growth with optimum oxygen tension. Therefore, certain strains of bacteria tend to grow in those tissues which afford just the right grade of oxygen tension. This has been demonstrated by E. C. Rosenow. Perhaps other chemical properties of the bacteria, or chemical properties of the tissues of the host, may aid materially in the selection of a site in the body by the bacteria.

The determining factor of greatest moment in gaining a foothold by bacteria in any particular location is the number of bacteria reaching and sticking in that spot.

The experiments of Krause have beautifully demonstrated the part that the lymphatics play in centralizing an attack of bacteria on a lymph node or nodes, centralward from where they were first introduced into the body; or, after the bacteria have reached the blood and the blood passes through the lungs, many of the bacteria are filtered out, passed into the lymph spaces, and on to the bronchial lymph nodes. In the experiments of Rogers, in which guinea pigs were submitted to massive doses of tubercle bacilli, some of the inhaled bacteria stuck in the lung parenchyma, although most of them doubtless must have passed on to the lymph nodes. The lymphatics evidently were unable to transport all the tubercle bacilli before some gained a foothold.

The selection of a growing site by bacteria depends, next to the number of bacteria making the attack, upon the condition of the lymphatic system. If it can be shown that the lymphatic circulation in the apices is obstructed, or hindered markedly, above what exists in the rest of the lungs, we shall have a logical explanation of apical tuberculosis.

A number of reasons have previously been advanced for a relatively slow lymph circulation in the apices. An analysis of the literature, as presented in the preceding paragraphs, gives us the following:

1: Since the circulation of blood and lymph throughout the body, especially throughout the lungs, is markedly facilitated by the respiratory movements and since the apices of the lungs make lesser excursions than do other parts of the lungs, the circulation of the lymph and blood must be accelerated by the respiratory movements less in the apices than in any other part of the lungs. The apices are relatively immobile because of the narrowness of the pleural cavity of this section, and because they are so far removed from the diaphragm that the enlarging of the pleural cavity downward does not affect the apices. Shortened first rib cartilages and malformation of the ribs and vertebrae may reduce the upper thoracic aperture and further reduce the mobility of the apices. In

case the first rib cartilages are ossified, the mobility of the apices would be still further reduced. The ossification of the cartilages is a factor more likely to prevail in adulthood than in childhood.

2: The rapid growth of the lung, as pointed out by Pottenger, relatively greater in the apices as compared with the thorax containing the apices than the rest of the lung as compared with the thorax containing it, causes an undue compression of the apices; this causes smaller spaces for air, lymph and blood. The lymph stream being of low pressure, a narrowing of the spaces and channels has a decidedly greater influence on the lymph circulation than on either the blood or air circulation. The compression of the apices, as a result of relative overgrowth, is a factor that does not exist in early childhood.

3: The departure of the upper ribs from the horizontal after the child assumes the erect position, as explained by Pottenger, adds another factor to compress the lungs and narrow the lymph spaces in the adult, which is not present in early childhood.

4: The peculiarly dwarfed appearance of the apical bronchi, discovered by Birch-Hirschfeld, would indicate short, kinky bronchi, and hence angular, kinky lymphatics, through which the circulation of the lymph would be impeded by the shape of the vessels. This element has not been previously suggested, as far as I know, to explain apical tuberculosis.

5: The deposition of dust, soot, etc., within the lymph spaces and vessels of the apices, would probably be no greater than in other portions of the lungs; but the fact that there is a slower lymph circulation in the apices than elsewhere would render the foreign material less likely to be swept on from the apices to the bronchial nodes and past these; as the dust, soot, etc., gradually accumulate in the apical lymphatic vessels and nodes these would inhibit the apical lymph circulation. The dust element would scarcely become a factor before adulthood, unless the child were living in exceptionally dirty atmosphere for long periods.

6: What has been said of dust applies identically to bacteria of all types that might be inspired. The bacteria would have, in addition to a mechanical blocking, an opportunity for their toxin to produce chemical insult; mildly inflammatory or even pneumonic attacks might result in further injury. We now realize, as never before, that bacteria may lie dormant, or nearly so, in tissues for protracted periods with no noticeable effect, and yet there are changes (injury) being persistently produced. The nodes would likely suffer more than would the vessels. The bacterial element would be accumulating with the years and hence mainly operative in slowing the lymph circulation in the adult.

7: The furrow made about the apices, as pointed out by Schmorl would cause atelectatic areas, with compressed, narrowed, angular and perhaps injured lymph channels. Shaw has already demonstrated that a collapsed lung will be the site of election for tubercle bacilli circulating in the blood; dust should also gather most plentifully in a collapsed lung. A small area of collapsed lung, as must exist under Schmorl's furrow, offers a favorable site for the locating and sticking of tubercle bacilli. This element prevails more in the adult than in the child.

8: That the orthograde posture has the influence attributed to it by Phillips in producing anemia of the apices as compared with the other portions of the lungs, I am inclined to doubt; but if the apices are robbed of blood by the upright posture, in which man spends the greater portion of his time, the lymph would be proportionately less in the apices, and the danger of infection, at least from air-borne bacteria, proportionately greater. Nature has made wise provision in the vasomotor control to keep an equitable adjustment of blood to a part, irrespective of posture. Otherwise, man would lie horizontally, or better with head low, instead of pacing the floor, in order to think. In some cases, however, the orthograde posture may render the apices relatively anemic. Presumably it would be a pathological condition which would tend to allow the orthograde posture to produce anemia, and hence more operative in the adult than in the child.

9: The transference of infection from the supraclavicular lymph nodes to the lung pleura by some more or less direct route, as claimed by Van Zwaluwenburg and Grabfield, may be an additional important factor contributing to apical tuberculosis. I have been unable to find an explanation of the channels by which bacteria might get directly from the supraclavicular lymph node to the pleura of the apices and feel that I am unable to criticize this theory. If bacteria do reach the pleura in this way, they must be fed to the upper bronchial node and hence would offer an additional insult to apical lymphatics and nodes. I predict that these workers will find some other way of explaining the development of the "pleural cap." This would be another factor more operative in the adult than in the child.

10: I desire now to add as the tenth factor for an obstructed lymph circulation the communication between the deep cervical lymphatics, the mediastinal nodes and the tracheobronchial nodes.

The portal of entry of practically all foreign particles entering the body is the mouth and nose. The mouth and pharynx, with the teeth,

gums, tonsils, adenoid tissue and the nose (with its narrow cavities easily occluded) and the accessory sinuses, offer locations in abundance for the accumulation and incubation of the bacteria which are constantly reaching these sites. The cervical lymphatics are constantly subjected to an assault of bacteria and other substances from the mouth and nose; the lymph nodes in the thorax which are anastomosed with the cervical lymphatics are in turn susceptible to assault from the same bacteria and virus and also from those which develop in transit. The deep cervical lymphatics drain into or communicate with the mediastinal nodes and these have communicating lymphatics to the bronchial nodes. The material drained from the bacterial nests of the head and neck, by more or less direct channels, then may reach the bronchial lymph nodes. The topmost node of the bronchial group usually would receive a greater number of bacteria from the cervical lymphatics than would any of the lower nodes. The swollen topmost bronchial node might have a disastrous influence upon the apex, as will be pointed out later.

The bronchial nodes are under conditions unlike any other in the body. The lung in any plane is fan-shaped; the arteries, nerves and bronchi going into the lung form a compact mass at the hilum and there occupy a small space; the nerves, arteries, lymphatics, etc., rapidly spread out, branching tree-like throughout a relatively large area in the lung. Ordinarily, the enlargement of a lymph node or two of any particular chain has little or no tendency to produce a stasis of the lymph in the drained area. Whatever leaves the lung is supplied and collected from a large area to pass through a relatively small space at the hilum. Hence, contrary to the general rule in other parts of the body, a swelling, even slight, in the node, or about it or the hilum lymph vessel, might exert a sufficient pressure to interfere with the outflow of the lymph stream coming from the lung apex under low pressure.

While all the bronchial nodes have a tendency to become inflamed, those draining the apices somewhat more so than those draining the rest of the lungs, from the bacteria which are filtered from the blood capillaries and passed on to the nodes, the assault of bacteria from the cervical lymphatics which is made upon the topmost bronchial node, causes an excessive swelling of the node draining the lung apex and hence may produce a stagnation of lymph in the apex. The stagnant lymph consequently forms a good growing place in the apex for the bacteria. In rare instances, the infection that reaches the bronchial nodes from the deep cervicals is shunted to another node than the topmost node of

the hilum and the area of the lung to be affected will then be the central portion or even the base.

In children there has not been sufficient time for the respiratory tract to become infected with bacteria and bacteria nests to the extent that will cause serious swelling of the bronchial nodes and an interference with the apical lymph circulation.

An X-ray plate shadow of an indurated area in an adult lung is usually conical with the apex of the cone pointing downward toward the hilum; or perhaps the cone has its base in the lower lobe of the lung, with the apex of the cone pointing upward toward the hilum. The apex of the cone is directed toward a lymph node. Crane (45) calls attention to "the inverted comma sign in lung X-rays." The head of the comma, which is about the size of a pea, is at the level of a bronchial bifurcation; the tail tapers upward along the bronchial border for 2 to 3 cm. and fades into the shadow of the bronchus. Crane says that this comma shadow is an evidence of chronic tuberculosis of this area, probably indicating an area unusually susceptible to tuberculous infection; he thinks that it is connected with a single lymph node.

Tubercle bacilli may reach the bronchial nodes from tuberculous tonsils or other tuberculous foci about the mouth, nose, throat or head; and they may assist appreciably in causing obstruction of the node and stagnation of the apical lymph, and the tubercle bacilli might conceivably grow up the lymph channels containing the stagnant lymph; but it is more likely that if these same tubercle bacilli are to attack the apical tissue they first get into the blood and then are filtered from the blood through the apical capillaries and thus gain entrance to the parenchyma of the apices. Tubercle bacilli from the air or food may in this way be the source of tuberculosis of the apices. If the lymph node, draining the apical area now infected with tubercle bacilli, has not previously been infected with tubercle bacilli it now will become infected.

This accounts for Ghon's (46) observations that a tuberculous lesion of the lung tissue is older than a tuberculous lesion of the node. It was his contention that tuberculosis was primary in the lung tissue and secondary in the node. The node may be diseased and swollen from pyogenic microörganisms, to the extent that the lymph flow from the apex becomes extremely sluggish. If, then, tubercle bacilli come by way of the blood, they are taken up by the lung cells and, the lymph being sluggish and unable to carry them on, they grow in the lung and later get to the node.

The course of reasoning which I have presented would necessitate that the early part of the process, the preparation of the soil, as it were, for the implantation of the tubercle bacilli in the lung tissues, is usually accomplished by the ordinary pyogenic bacteria. Other reasons for this conception may be cited: 1. The history of many cases of pulmonary tuberculosis indicates that for months or even years before tuberculosis was proved to exist, or perhaps was even suspected, there were frequent "colds," protracted "colds," sore throat, nasal catarrh, bronchitis, etc., at first in winter only but finally bridging from spring through summer and fall to winter. 2. In the incipient stage of pulmonary tuberculosis it is not infrequent that most diligent search fails to reveal tubercle bacilli in the sputum and sometimes, even in what appears to be advanced tuberculosis of the lungs, the tubercle bacilli may not be found. 3. Tubercle bacilli are slow growing in the culture tube, in the laboratory animal, and in the human body, as compared with the more ubiquitous pyogenic nose and throat bacteria. According to my course of reasoning, then too, the finding of tubercle bacilli in the sputum may be in reality an index of the severity of the pyogenic catarrhal infection of the lung.

If my reasoning is correct, many of the so called typical X-ray shadows in lung plates may in reality be of processes produced more by the pyogenic catarrhal germs than by the tubercle bacilli.

My conclusions are that there are a number of factors operating to effect apical tuberculosis. They all have a primary or secondary effect of injuring the apical lymphatics or impeding the lymph circulation of the area.

The relative lack of mobility, the narrow upper thoracic aperture, the shortened and firm first costal cartilage, the possibly ossified first cartilage, the dropping down of the sternal ends of the first ribs, the furrow made by the first rib, the malformed vertebrae, the dwarf-like apical bronchi, the relatively overgrown apex, the gradual accumulation of dust and bacteria in the apical lymphatics, all individually or collectively may play a part in helping to centre tuberculous infection in the apices.

The compression of the lung apex which results from the various factors causes a partial collapse or crowding of the lung tissue, and in turn an interference with the lymph circulation, and is certainly a more important factor in causing apical tuberculosis than is lack of movement.

However, the paramount determining factor in the selection of the apices by pulmonary phthisis is, it seems to me, the long continued assault, which occurs in all adults, to a greater or less degree, upon the topmost bronchial lymph nodes by bacteria and their toxins. The germs and their poisons are picked up by the lymphatics from the nose and mouth and the entire head, and passed on by the lymphatics of the source of the infection to the deep cervical nodes and thence to the mediastinal and the bronchial nodes. The swelling of the bronchial lymph node dams back the lymph into the area drained; since it is the topmost bronchial node which usually is affected most seriously, the resulting stagnant lymph will be in the apex of the lung, where already the circulation of lymph by virtue of the relative immobility of this part of the lung is at a low ebb. In this way there is produced a fertile soil for tubercle bacilli coming from whatever source they may.

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CORPORATION CARE OF TUBERCULOUS EMPLOYEES

AN EXPERIMENT AS CARRIED OUT IN COLFAX BY A LARGE CORPORATION[1]

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When the president of our Association asked me to present a paper it occurred to me that probably the most appropriate topic to bring before an association whose members act as medical advisers and surgeons to railroad corporations, would be the outlining of the plan adopted by one of the large corporations of California for the care of its tuberculous employees. I felt that you would be interested not only in the plan itself but also in the results obtained. In going over with you the scheme adopted by this corporation and in also reporting the results so far obtained, it seemed to me that it would be very fitting to express to your body some ideas which I have had for a great many years as to how it would be possible to increase very materially the number of arrested cases turned out by our institutions and also how to prevent the relapse of such discharged individuals.

I have felt that many patients have needlessly lost their lives and that these lives could have been saved if certain factors could have been controlled. It has always seemed to me that the two most discouraging things in the treatment of tuberculosis were first, those patients who are doing well and should have an excellent opportunity of securing an arrest of their disease become restive after they have lost many of their symptoms and want to return home and frequently do return home too early and eventually succumb to tuberculosis. They do not realize the great seriousness of tuberculosis, nor do they realize how long it takes before it is safe to return home. They do not grasp in the slightest degree how long it will take before they are able to work. The other discouraging thing is that those patients for whom one can do nothing and who might as well be at home, where they will be with their families and where they will be spared the expense of additional treatment, wish

[1] Read before the Pacific Association of Railway Surgeons, San Francisco, August 25, 1922.

to remain indefinitely. This is a very serious thing because the number of beds for tuberculosis patients is limited, and not infrequently a patient who has a chance to get well is unable to avail himself of institutional treatment because so many beds are held by patients who cannot get better.

How to increase the percentage of arrested cases and bring the number of nonarrestable cases to the irreducible minimum is something to which every serious student of tuberculosis gives much time and thought. It has always seemed to me, and I have publicly expressed this thought on numerous occasions, that the two most important factors in securing an arrest of tuberculous disease are rest and time. If all patients suffering from tuberculous disease can be given rest or modified rest under proper medical supervision over a sufficient length of time, the number of permanent arrests could, I believe, be increased at least fifty per cent. That this belief is based on a foundation of fact has been amply proved, to my satisfaction at least, by my experience with patients who have remained in Colfax, either in my employ or in the employ of the various units of the institution. We have to-day working for us, either for me personally or for the managements of the different institutions, a number of patients who came to us in a very advanced condition with very extensive lesions, not only pulmonary but in some cases pulmonary and laryngeal, who now for a number of years have been working steadily and who have lost through sickness very little, if any, more time than the average nontuberculous employee. I have felt that the reasons for the recovery of these very advanced patients have been:

First, they have taken rest or modified rest over a long period of time.

Second, they have continued a modified regimen for a long period of time.

Third, they have commenced with very light duties, never at any time exceeding their physical limitations, and have gradually increased the amount of work and responsibility as their limitations would allow them.

Fourth, they have considered themselves patients first and employees afterward.

For many years I have planned or tried to plan for some institution, where I could have absolute control, not only of the patients' regimen but also of the length of time that the patients must stay in an institution, and where in addition I could have supervision or at least the position of adviser regarding the length of working hours and the character of the work which the patient will assume after his discharge from the

institution. I have felt, if an opportunity of this sort could be afforded me, that without doubt I could raise the percentage of permanently arrested cases very materially. An opportunity to try out this scheme has been afforded me within the past two years by one of our large corporations, the Standard Oil Company of California.

Dr. Louis P. Howe, the Chief Surgeon of the company, about two years ago commenced a systematic examination of all employees, in order to discover correctable defects, medical or surgical, which might be present among the company's employees. Wherever defects were discovered it was the policy of the company to endeavor to correct them. As a result of this examination, it was found that not the least of the problems presented was what to do with those employees who were found to have active tuberculosis. After consultation, it was decided to erect an institution known as the *Standard Oil Company Sanatorium* for the care of such employees. It was agreed by the officers of the company that the objects of the sanatorium should be twofold:

First, to restore to work those whose disease had not progressed beyond the hope of cure, and

Second, to furnish medical and nursing care and hospitalization for life, to those employees for whom, because of the extent of their lesion, there was no hope of recovery.

With these objects in view the following regulations were adopted:

First: Every tuberculous employee would be offered the opportunity of entering the company's sanatorium at Colfax.

Second: All patients taking advantage of this opportunity were to be absolutely under the control of the medical superintendent of the sanatorium. This meant that patients would be required to live up to the rules of the medical superintendent and also that they would, of necessity, remain in the institution until such time as he should deem it safe to allow them to return to work.

Third: No patient should be discharged until he was well enough to undertake light work.

Fourth: The medical superintendent should prescribe the character of work which the patient should assume after leaving the institution and would also prescribe the number of working hours per day for each patient. This would allow an opportunity to work out my belief as to the value of rest and time, combined with supervision, both in the sanatorium and during the time subsequent to discharge.

The Standard Oil Company Sanatorium was opened in August of last year, but previous to this time patients had been treated in other of the different units of the Colfax School for the Tuberculous, so that the experiment began in November, 1920, rather than in August, 1921.

The treatment afforded patients has not been markedly different from that used in all well-conducted modern sanatoria for the treatment of the tuberculous. The only difference between the method of handling the patients in the Standard Oil Company Sanatorium and the method used in the treatment of the patients in the other units was that the patients were required to remain until the medical superintendent deemed it wise to allow them to leave and, also, that their living and working conditions following discharge from the sanatorium were supervised. All febrile patients were put to bed and have been kept as bed patients until the temperature has been normal long enough to allow time up. The matter of allowing time up and exercise has been the same as in the other units. Tuberculin has been used for certain patients, stock vaccines and autogenous vaccines for others, artificial pneumothorax in those cases where it was indicated, and heliotherapy both by direct rays of the sun and by means of the Alpine sun lamp. In none of these things has the treatment been different than in the other units.

From November, 1920, to August 1, 1922, there have been entered 64 patients. These have been classified as follows:

Early cases	17
Moderately advanced	19
Very advanced	26
Diagnosed as nontuberculous	1
Diagnosis not completed	1

Among these 64 patients there were 28 with complications. These complications included:

Pleurisy with effusion	4
Tuberculous otitis media	1
Labyrinthine disease?	1
Gastric ulcer?	1
Ischiorectal abscess	3
Hemorrhages	3
Lues	3
Genitourinary tuberculosis	2
Tuberculous peritonitis	3
Tuberculous laryngitis	6
Glandular tuberculosis	2

Cerebrospinal lues	1
Tuberculosis of spine	1
Tuberculosis of sternum	1
Tuberculosis of right ilium	1
Bilateral pleural effusion	1
Bronchial asthma	1

Among the 64 patients 29 have been discharged and have returned to work. The average length of time that these 29 patients remained before they were able to return to work was seven months. The maximum time was twelve and one-half months and the minimum, one and one-half months. Of the remaining 35 patients, 5 have died, 26 are still under treatment, 1 was discharged as nontuberculous and 3 others were discharged for other reasons. Of the 5 who died, the average number of months in the institution was three and one-half, the maximum being five and one-half and the minimum one and one-half months. As can be readily seen from the short period of hospitalization, these patients were all very advanced at time of admission. The average stay in the institution of those 26 patients who are still under treatment is nine months. Among these 26 there has been an average gain in weight of $15\frac{1}{4}$ pounds, the maximum being 40 pounds and the minimum three-quarters of a pound. Only 1 of these patients has lost weight and he has lost 3 pounds.

You will note that the average stay in the institution of these 26 patients is already nine months, which is two months longer than the average stay of those who have been returned to work. This is explained by the fact that these 26 include some very advanced patients who have remained a long time under treatment. There are in the institution at present 15 patients who have been under treatment eight months or more, and 10 of whom have been hospitalized for twelve months or more. Seven have been in the institution less than three months. Of the 15 patients who have had more than eight months' hospitalization all but 3 are afebrile. The average gain in weight of these 12 afebrile patients has been 20 pounds. All of them, with one exception, are allowed up several hours a day and are on graduated exercise and 3 of them have been recommended as ready to return to work when light occupations can be provided for them. The 9 others who are allowed time up and exercise are, in my opinion, in spite of their good general condition, still not well enough to be allowed to return to work because of the danger of a relapse.

To return to the 29 patients who have been discharged and have returned to work. These were classified on admission as

Early cases	11
Moderately advanced	12
Very advanced	6

They have now been working an average of six months. The maximum length of time that any one individual has been working is fifteen months. The last report from all of these patients has been that every one is as well or better than at the time of discharge, and is working every day.

As stated above, 3 other employees among those at present in the institution have been recommended for light work, which will bring the number of patients working to 32, or 50 per cent of 64 admitted. The average stay of these 3 patients in the institution is seventeen and one-third months.

In order to see at a glance the results of the work so far accomplished I have made the following table, showing the results to date of treatment of the 64 patients admitted:

Patients admitted to date	64
Patients discharged and at work	29 or 45.4 per cent
Patients discharged as nontuberculous	1 or 01.5 per cent
Patients discharged for other reasons	3 or 04.7 per cent
Patients who have died	5 or 07.8 per cent
Patients ready to return to work	3 or 04.7 per cent
Patients very much improved	7 or 10.9 per cent
Patients improved	14 or 21.9 per cent
Patients unimproved	2 or 03.1 per cent

There is one very important item which should be mentioned, in closing, which helps in a large degree to explain the good results obtained in the Standard Oil Company Sanatorium, and that is the matter of financial assistance. As stated above, it is the opinion of the author that the two most important factors in the cure of tuberculosis are rest and time. The ability to take rest over a sufficient length of time, in order to secure an arrest of tuberculous disease, depends in the last analysis upon ability to finance the cure. Financing the cure means several things:

First, the ability to pay the cost of hospitalization and medical care.

Second, the ability to finance the care of the other members of the family at home.

Third, the ability to finance the home following the discharge of the patient from the Sanatorium.

A patient who has to worry continually about these three items is handicapped from the start. The patient from whose mind the financial burden has been lifted faces the many months of cure in a much more cheerful frame of mind and has much brighter prospects for eventual success. Even where the task of raising the necessary money is not an insuperable one, the discharged patient in ordinary life meets a very serious problem on returning home. He looks well, feels well, eats well, sleeps well and is apparently in the best of health. At the institution from which he has graduated he has learned his physical limitations, just how much he can do and what he cannot do. He has been instructed in the number of months it will be before be can begin work, or, if ready to work, in the number of hours per day he can with safety perform his duties. He may go home absolutely determined to live up to the letter of the law as outlined for him in the sanatorium; but, because of the failure of his family, his friends and his employer to realize that he is still a sick man in spite of his healthy appearance, he is often considered as a slacker, as lazy or as a hypochondriac. Even though he is not directly accused of those faults, still he cannot help but sense the feelings of his associates. Too often his pride gets the better of his judgment, he returns to work too soon or he works too long hours, with the result that he sooner or later breaks down, instead of securing a permanent arrest of his disease. In the case of the employees discussed in this paper, these problems are solved for them. Each one knows, when he enters the institution, that his stay will be financed as long as it is necessary for him to remain. He knows his family will not be allowed to suffer the want of any necessity and when he returns home he is under the supervision of the Company officials and is not allowed to work more than the limit prescribed by his physician. Any one who has had the care of tuberculous patients can see at a glance what a tremendous advantage such an employee has over his fellows.

The problem of handling tuberculosis cases successfully is a tremendous one. If we merely attempt to prolong life or make patients comfortable or to control infection by isolation of patients in sanatoria, we only partially solve this problem. What we must attempt to do is to get as large a percentage as possible of nonproducers again into the producing class. It is quite possible to materially raise the percentage of arrested cases, provided we can have control over the patient and his

activities for a sufficient length of time, not only while he is in the institution but also after he leaves the institution. In order to attain these ends we must have not only the thorough coöperation of the patient but also that of the employer and, in addition, each patient must have sufficient finances or financial backing to carry out a scheme similar to the one outlined above. The greatest burden naturally falls upon the middle class, especially salaried employees. Numerous plans have been put forward at various times to meet the needs of just this class of individuals. Among the plans suggested and tried out in this and other countries have been health insurance and municipal, county and state sanatoria. I believe the plan conceived by the Standard Oil Company of California, as at present carried out at Colfax, is a distinct advance over other methods, and I believe that the results definitely prove the superiority of the methods employed by this corporation. To know just how useful the plan really will be, will, of course, take several years to determine, but I believe the results so far obtained amply justify the thought, the time, the energy and the money expended in the work.

THE RETICULUM OF THE LUNG

I. ITS RELATION TO EARLY TUBERCULOUS PROCESSES[1]

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Histologically, three types of connective tissue fibres, collagenous,[2] elastic, and reticulum, can be recognized as entering into the framework of a given organ, be it the lung, the liver, the spleen or a lymph node. Each member of this group of fibres has a special function to perform.

Collagenous fibres are arranged in bundles or spread out into membranes, which are of sufficient strength to resist unequal pressure or stretching.

Elastic fibres branch and anastomose, and by this arrangement form networks which are capable of stretching under tension and of returning to their normal position independent of any bodily exertion.

Reticulum is made up of fine branching and anastomosing fibrils that form basement membranes and, in the interior of an organ, enclose within its meshes the cells which make up that organ. The network of reticulum is quite distinct from that of elastic fibres and can be readily differentiated by digestion methods, and by selective methods of staining. Because of these characteristics reticulum has been placed in a class by itself. It will be shown, however, that it bears a very definite relation to the bundles of collagenous fibres.

In the present study the relation of reticulum to the early stages of the tuberculous process will be discussed, leaving the later stages of tuberculosis and the general description of reticulum for future communications. All the photomicrographs used in illustrating this paper are taken from sections of human lungs. No use has been made of tissues of experimental animals.

[1] Aided by a grant from the National Tuberculosis Association.

[2] I prefer the term *collagenous* to the older one of *white fibrous connective tissue* because it is shorter, and because, chemically, the fibres consist of the albuminoid *collagen* which yields gelatin when boiled in water.

A single tubercle[3] may or may not be built up around a giant cell. In the former case it consists of a central part which is occupied by a giant cell containing a large number of nuclei that are arranged in a variety of groupings near its periphery. Surrounding the giant cell is a variable number of layers of epithelioid cells and lymphocytes, between which the protoplasmic processes of the giant cell ramify (fig. 1). The whole structure is held together by a system of branching and anastomosing fibrils—the reticulum. In the second case it is made up of epithelioid cells and lymphocytes which are held together by a network of reticulum.

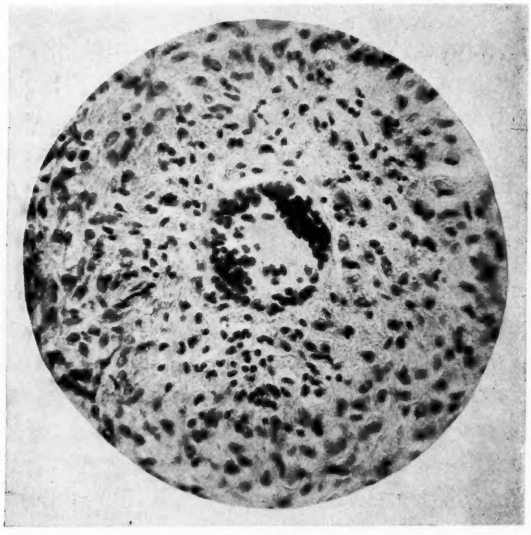

FIG. 1. A SINGLE MICROSCOPIC TUBERCLE CONSISTING OF A GIANT CELL, CONTAINING NUMEROUS NUCLEI AND SURROUNDED BY EPITHELIOID CELLS AND LYMPHOCYTES.[4] × 200

The history of one of the lungs from which the sections used for the photomicrographs were made is of interest, because of its possible bearing on the transmission of tuberculosis from the mother to the child *in utero*. It is not necessary to enter into full details of the case; the essential facts are included in the following statement:

During the latter part of gestation the mother developed an active tuberculosis. Shortly after birth the child came into a Cleveland, Ohio, hospital

[3] By a "single tubercle" I mean a single microscopic tubercle before there is any fusion with other like tubercles to form a conglomerate tubercle.

[4] The use of a strong reading glass will materially assist in the study of all the photomicrographs.

with a well marked miliary tuberculosis of the lungs. It died before it was four months old. An autopsy was made a few hours after death and, in addition to the pulmonary tuberculosis, a tuberculous meningitis was found. The lungs were filled with 95 per cent alcohol and the following day the left lung was sent to me. The entire left lung contained scattered tubercles ranging in size from a pin point to a millimetre in diameter.

In order that the following descriptions may be easily followed, and understood, the technique of the preparation of the sections will be given at the beginning rather than at the end of this study.

Blocks 0.5 cm. in thickness were cut from selected parts of the lung and imbedded in paraffin of 58° melting point. In every instance a ribbon of 150 to 200 sections was cut and the sections were mounted on three sets of slides in such a way that, for example, sections 1, 4, 7, 10, 13, were mounted on slide one; sections 2, 5, 8, 11, 14, were mounted on slide two; sections 3, 6, 9, 12, 15, were mounted on slide three. This arrangement of sections was continued until the required number had been mounted. Each set of sections was stained by a different method; set one was stained with haematoxylin and eosin; set two was stained with a modification of Bielschowsky's stain; set three was stained either with Weigert's resorcin-fuchsin and counterstained with Orange G, or with my modification of Unna's orcein stain with ammonium picrate for a counterstain. In some instances Mallory's connective tissue stain was substituted for Bielschowsky's stain. The sections were cut either 7 or 10 micra in thickness and fixed on the slides with sterilized distilled water. By using this method of mounting and staining the sections it is easy to recognize the relation of reticulum and elastic fibres to the structures under observation, there being either 14 or 20 micra between every three sections depending on the thickness they are cut.

In addition to this, blocks were digested in pancreatin, washed carefully in water, and passed slowly through the various grades of alcohol into chloroform. They were then imbedded in paraffin of 52° melting point and cut serially into sections of various thicknesses. Sections 40 to 60 micra thick gave the best results. These sections were either stained with Mall's stain for reticulum or with nigrosin.

As the result of the study, from an anatomical standpoint, of the material used in this investigation, I have found a definite increase of reticulum in typical tubercles associated with the formation of giant cells. The details of distribution of this newly developed reticulum will be apparent from the descriptions and photomicrographs which follow.

Coincident with the development of a giant cell there is an increased development of reticulum, which forms a basket network of branching

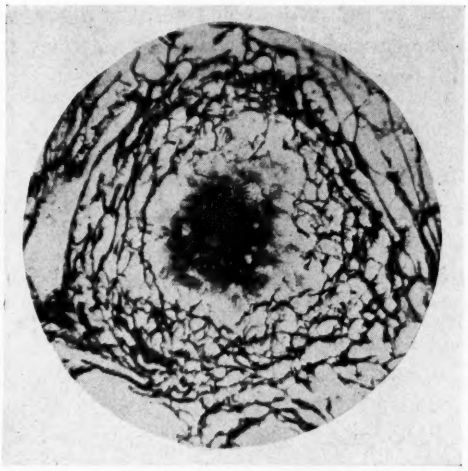

FIG. 2. A SINGLE MICROSCOPIC TUBERCLE WITH ITS CENTRAL GIANT CELL ENCLOSED WITHIN A BASKET NETWORK OF RETICULUM. × 200

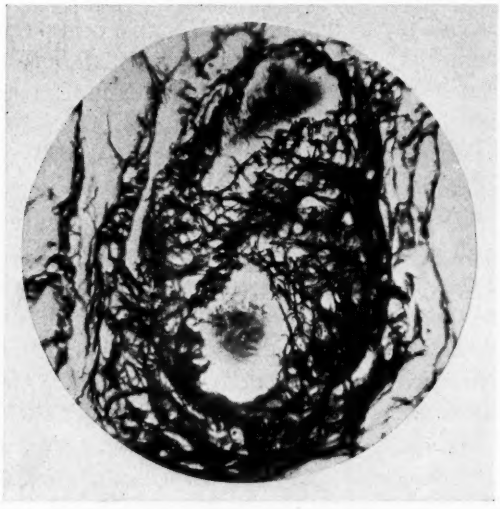

FIG. 3. TWO GIANT CELLS ENCLOSED WITHIN A COMMON NETWORK OF RETICULUM. × 200

and anastomosing fibres about the giant cell (fig. 2), and encloses within its meshes the epithelioid cells and lymphocytes. The size of the fibres of reticulum varies greatly. In figure 2 those which immediately cover

the giant cell are much finer than those on the periphery of the cell and still coarser fibres are seen surrounding the entire tubercle. When two giant cells are enclosed within the same basket network of reticulum or when the network about two giant cells fuses, the resulting fibres are much coarser. Thus, the network which surrounds the two giant cells in figure 3 is composed of much coarser fibres and the mesh is much denser than in figure 2.

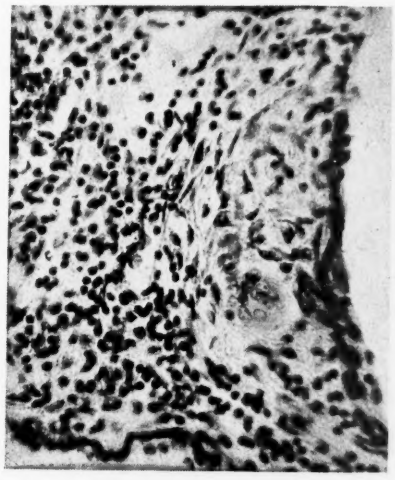

FIG. 4

FIG. 5

FIG. 4. A TUBERCULOUS PROCESS SITUATED BETWEEN A DUCTULUS ALVEOLARIS AND THE PLEURA

Note the giant cell just outside the wall of the ductulus. × 200

FIG. 5. SECTION IMMEDIATELY FOLLOWING FIGURE 4, SHOWING THE RETICULUM

The network of reticulum lying next to the lumen of the *ductulus alveolaris* belongs to the giant cell, while the reticulum of the blood vessel is marked out by the parallel lines just above it. × 200.

The presence of the network of reticulum about a giant cell is often of assistance in locating its position in respect to the surrounding structures. Figure 4 is a photomicrograph of a section through a subpleural tuberculous process, stained with haematoxylin and eosin, in which a giant cell is seen situated just outside the wall of a *ductulus alveolaris*. In the section, and in the photomicrograph, the cell appears to be situated within a blood vessel; but if the succeeding section which was stained by Bielschowsky's method and is only 10μ removed, be carefully

examined, the network of reticulum about the giant cell can be made out quite distinct from the reticulum of the blood vessel which lies directly above and partially encircles it (fig. 5). It is interesting to note that Krause, working on experimental tissues, arrives at the same conclusion that the author of this article, working on tuberculous processes in the human lung, has reached: "We have never observed tubercle developing from proliferation of the intima nor in the vessel lumen."

A tubercle which has not yet come to involve the wall of a blood or lymph vessel, or of an alveolus, does not contain any elastic fibres. Repeated staining, with various special stains, failed to bring out any elastic fibres within such a tubercle; collagenous fibres were also absent.

There are, however, formations made up originally of single tubercles, which arise separately but very close to each other, which, by their fusion, form those larger or smaller masses that are called miliary tubercles; but, because they are made up of a number of single elements, they are better designated *conglomerate tubercles*.

With the coalescence of the single tubercles to form a conglomerate tubercle certain changes take place. The resulting conglomerate tubercle is no longer a round mass but it becomes lobulated or "mulberry shaped." Its centre soon changes its character; the cells disintegrate and sooner or later caseation gradually takes place. These changes may also begin in the single tubercles before fusion occurs.

Figures 6, 7 and 8 are photomicrographs of consecutive sections through a conglomerate tubercle. Figure 6 shows the characteristic mulberry appearance of the caseous centre surrounded by epithelioid cells and lymphocytes. Some of the more peripheral alveoli exhibit exudative features of the tuberculous process, while others present an emphysematous dilatation. In figure 7 the reticulum is stained. The central portion, which in the preceding section appears to be a structureless mass, in reality contains a large amount of reticulum. Two giant cells that are not distinguishable in figure 6 appear well defined and are surrounded by a network of reticulum which merges into that of the tubercle. The darkly stained areas represent groupings of lymphocytes and epithelioid cells which are undergoing caseation. On the right of the photomicrograph and partially surrounding an open space, a bundle of collagenous fibres appear. That the network of fibres in figure 7 is *not* made up of elastic fibres is shown in figure 8. At this stage of development of a tubercle the amount of reticulum far exceeds that of elastic fibres; the few elastic fibres present are associated with the walls of the alveoli and of the blood vessels.

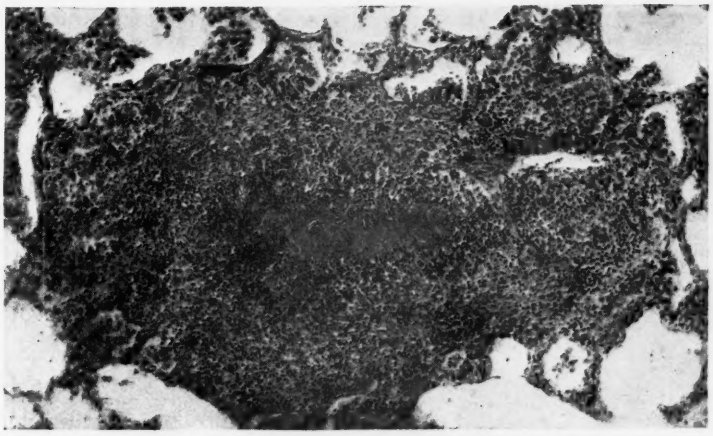

FIG. 6. A CONGLOMERATE TUBERCLE
Haematoxylin and eosin staining. × 75

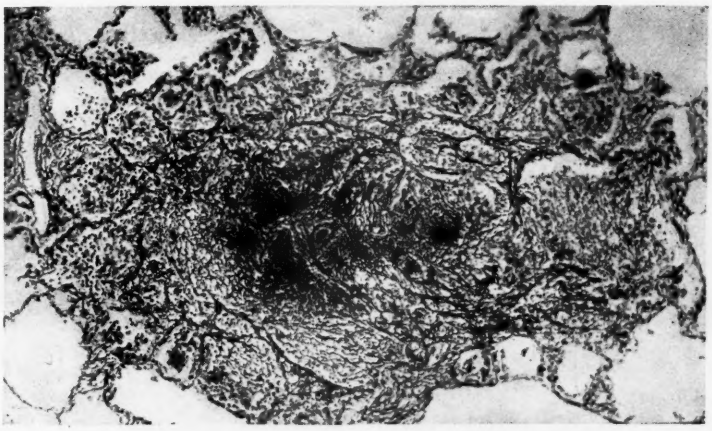

FIG. 7. SECTION IMMEDIATELY FOLLOWING FIGURE 6, WITH THE RETICULUM STAINED
Note the reticulum within the caseous centre. × 75

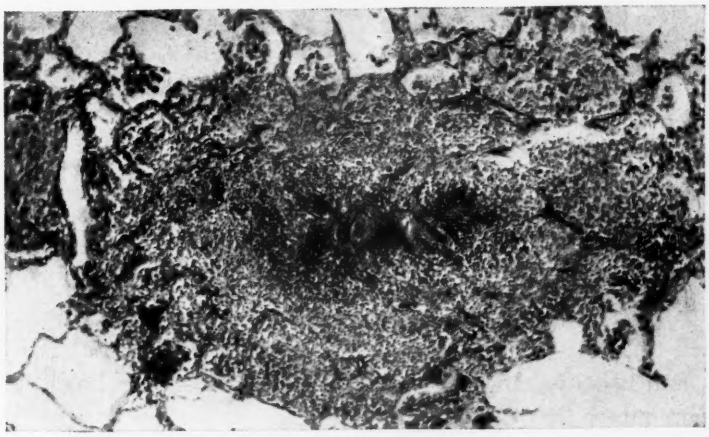

FIG. 8. SECTION IMMEDIATELY FOLLOWING FIGURE 7, WITH THE ELASTIC FIBRES STAINED. × 75

Figure 9 is a section through a bronchiole in which a tuberculous bronchiolitis is beginning to develop. The alveoli situated around its periphery are beginning to show the characteristic features of an exudative pneumonia. The exudate is similar to that within the alveoli surrounding the conglomerate tubercle shown in figure 6. A fragment of a giant cell can be seen in the upper right quadrant. The wall of the

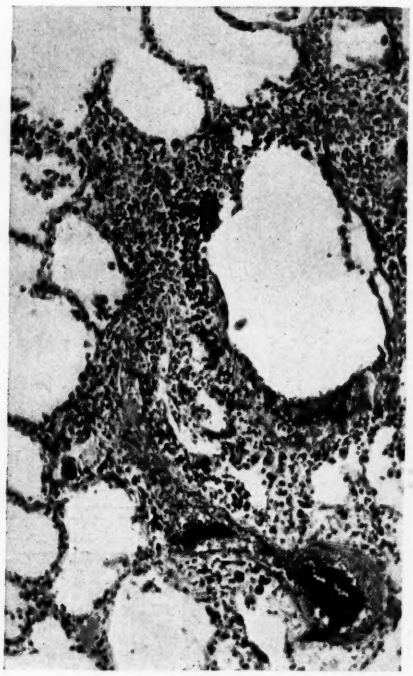

FIG. 9 FIG. 10

FIG. 9. EARLY TUBERCULOUS BRONCHIOLITIS AND PERIBRONCHIOLITIS
Haematoxylin and eosin staining. × 100

FIG. 10. SECTION IMMEDIATELY FOLLOWING FIGURE 9, WITH THE RETICULUM STAINED. × 100

bronchiole is being invaded by the tuberculous process and, although the lumen of the bronchiole appears nearly free, an exudate is beginning to be formed. This is more strongly emphasized in the following section (fig. 10). In this section the reticulum is stained; there is an increased quantity in the walls of the bronchiole and of the adjoining alveoli, and it is beginning to invade the interior of the alveoli in which the pneumonic process is the most advanced.

FIG. 11. EARLY TUBERCULOUS PROCESS IN A SMALL MASS OF LYMPHOID TISSUE ABOUT A BRANCH OF THE PULMONARY ARTERY

The plane of the section lies through a *ductulus alveolaris* at the place where three *atria* open into it. × 100.

This photomicrograph was taken with a lower magnification than figures 12 and 13 in order that the topography of the section could be shown.

FIG. 12

FIG. 13

FIG. 12. SECTION IMMEDIATELY FOLLOWING FIGURE 11; THE RETICULUM STAINED. × 200

FIG. 13. SECTION IMMEDIATELY FOLLOWING FIGURE 12; THE ELASTIC FIBRES STAINED. × 200

Figures 11, 12 and 13 are of more than passing interest because they show a tuberculous process taking its origin in the lymphoid tissue about a branch of the pulmonary artery. In a previous publication (1911) I have described the distribution of lymphoid tissue in the lung, and it was there stated that "when lymphoid tissue is present along the course of the pulmonary artery it is situated between the artery and the adjoining air spaces rather than between the artery and bronchus, as is the case with bronchial lymphoid tissue. The lymphoid tissue may extend for some distance along the artery, but the sheath-like arrangement described by some authors is not to be considered a normal arrangement."

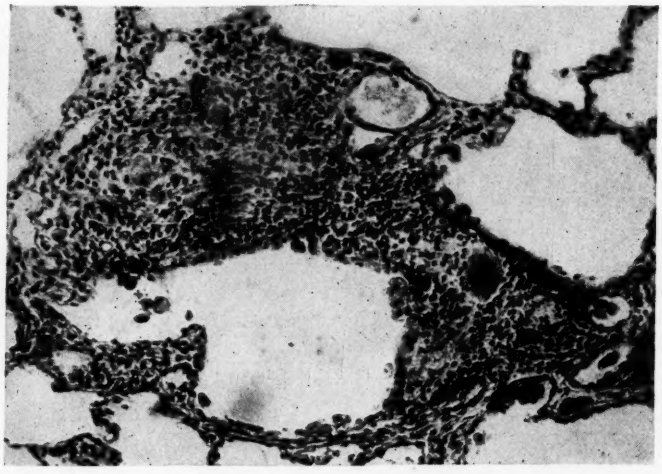

FIG. 14. TUBERCULOUS PROCESS IN A MASS OF BRONCHIAL LYMPHOID TISSUE

Note the peripheral situation of the artery. Haematoxylin and eosin staining. × 100

The amount of this lymphoid tissue is small and it does not become prominent until some irritant has been at work.

In figure 11 the artery is somewhat difficult to recognize, but when recognized it can be readily seen that the greater mass of the lymphoid tissue lies, not between the artery and the *ductulus alveolaris*, but between the artery and the adjacent air spaces. The distribution of the reticulum is shown in figure 12. The amount of lymphoid tissue is greater than normal and its reticulum has the characteristic arrangement of the reticulum of lymphoid tissue. Figure 13 shows the distribution of the elastic fibres. It also shows more clearly than figures 11 and 12 the increase in size of the mass of lymphoid tissue. Here, as in preceding

figures, the elastic fibres are associated with the blood vessels and the walls of the alveoli.

In contradistinction to figure 11, in which arterial lymphoid tissue is involved, figure 14 is introduced to show involvement of the lymphoid tissue belonging to a bronchiole. Here the artery can be seen at the periphery of the tuberculous lymphoid tissue which is situated between the artery and the bronchiole; the amount of lymphoid tissue is greater than in figure 11 and the reticulum is correspondingly increased.

FIG. 15

FIG. 16

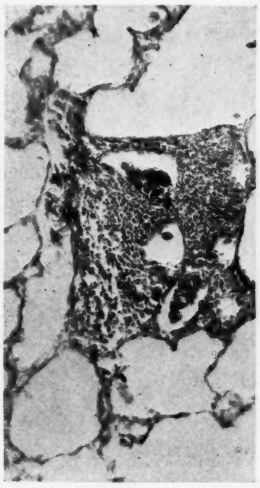

FIG. 17

FIG. 15. EARLY TUBERCULOUS PROCESS ORIGINATING ALONG A LYMPHATIC
Haematoxylin and eosin staining. × 100

FIG. 16. SECTION IMMEDIATELY FOLLOWING FIGURE 15; THE RETICULUM STAINED. × 100

FIG. 17. SECTION IMMEDIATELY FOLLOWING FIGURE 16; THE ELASTIC FIBRES STAINED. × 100

With the exception of the tuberculous changes illustrated in figure 11 all thus far described have been associated with giant cells. In figures 15, 16 and 17 the beginning of a tuberculous process along the margin of a dilated lymphatic, which is situated in a connective tissue septum, is shown. This process reminds one of what has been termed the "small cell tubercle" or the "lymphoid tubercle," and consequently resembles lymphoid tissue in its general structure.

In figure 15 lymphocytes form the greater part of the lesion, though here and there a polymorphonuclear neutrophile and an occasional

epithelioid cell can be distinguished. The succeeding section (fig. 16) shows the reticulum and how the tuberculous process is invading the adjacent alveoli. Figure 17 is a photomicrograph of the section immediately following figure 16 stained for the elastic fibres. It is nearer the centre of the tuberculous process, and shows how some of the alveoli seen in figure 16 have been nearly obliterated by the extension of the process. Had this section been the one to be stained for the reticulum,

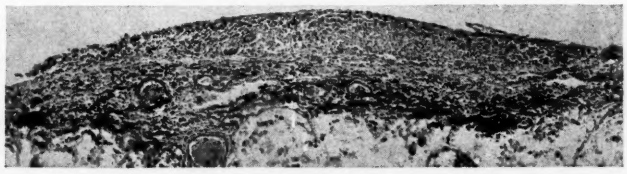

FIG. 18. TUBERCULOUS PROCESS DEVELOPING IN THE SUBMESOTHELIAL LAYER OF THE PLEURA
Haematoxylin and eosin staining. × 50

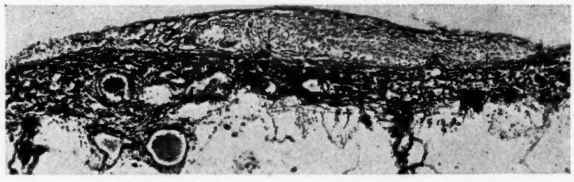

FIG. 19. SECTION IMMEDIATELY FOLLOWING FIGURE 18; THE RETICULUM STAINED
The heavy dark fibres in the pleura are not reticulum but *collagenous* fibres. × 50

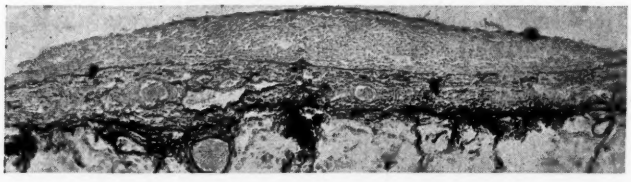

FIG. 20. SECTION IMMEDIATELY FOLLOWING FIGURE 19; THE ELASTIC FIBRES STAINED. × 50

it would have shown a much greater amount than figure 16. The few elastic fibres present are situated in the walls of the alveoli. Not infrequently there is found at the point where lymphatics unite a small amount of lymphoid tissue. It is not impossible that such was the case in this instance and that we have here an example of a tuberculous process developing in lymphoid tissue; for a careful examination of

figures 15, 16 and 17 will show that just above the tuberculous process two lymphatics unite.

A somewhat unusual type of a tuberculous lesion is illustrated in figures 18, 19 and 20. These sections are from the lung of a man thirty years old. Associated with the tuberculous process there was a pleurisy, which very likely was of tuberculous origin. No doubt the tubercle originated from an infection through the mesothelium covering the pleura. In figure 18 the distended pleural blood vessels are easily recognized and, between the section of the bronchial artery and pulmonary vein, a lymphatic, cut obliquely, can be seen. The plano-convex outline of the lesion contains two small giant cells. In figure 19 the tuberculous lesion is still more clearly defined. The heavy dark fibres and bands with somewhat hazy borders, seen in the pleura, are *not* reticulum, but are collagenous fibres, which in the stained section have a golden brown rather than a deep black color; on the other hand, within the tuberculous lesion the reticulum alone is stained.

In figure 20 the position of the lesion can be definitely located. The thin, dark line extending along the plane surface of the lesion represents the outer layer of elastic fibres which is presented in the normal pleura. What is situated outside of this forms the submesothelial layer and covering this layer is the mesothelium. The lesion is, therefore, confined to the submesothelial layer and has not extended into the underlying layers of the pleura, although a beginning lymphocytic infiltration is seen in figure 18.

The points of special interest in these sections are (1) there is no pulmonary lesion underlying the pleura, (2) there is no exudate or trace of adhesions over the lesion and (3) the lesion is confined to the submesothelial layer. While the three preceding figures illustrate a pleural lesion without any subpleural involvement, there may be present, on the other hand, a subpleural lesion without any pleural involvement.

In this study of the relation of reticulum to the early tuberculous process, use has been made of the lungs from some forty individuals ranging in age from four months to between forty and fifty years. A marked difference has been found in the amount of reticulum present in those processes in tuberculous lungs which are associated with giant cell formation and those in which, from the first, exudative pneumonia with early caseation has predominated.

If the pneumonic area about a growing tubercle (fig. 6) or if certain independent areas be studied, it will be found that the alveoli are more

or less completely filled with an exudate, in which shed alveolar epithelium, epithelioid cells and a few lymphocytes can be recognized, and that their walls are much thickened (fig. 21). If a section through such an area be stained for reticulum, the alveoli will be found to con-

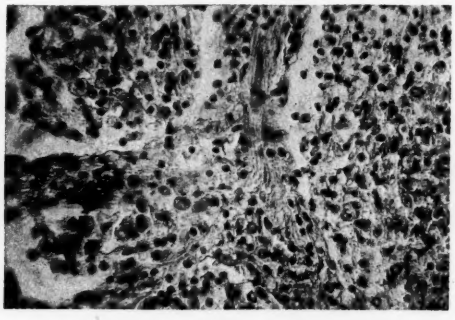

FIG. 21. TUBERCULOUS ALVEOLAR EXUDATE ASSOCIATED WITH THE FORMATION OF GIANT CELLS

Haematoxylin and eosin staining. × 200

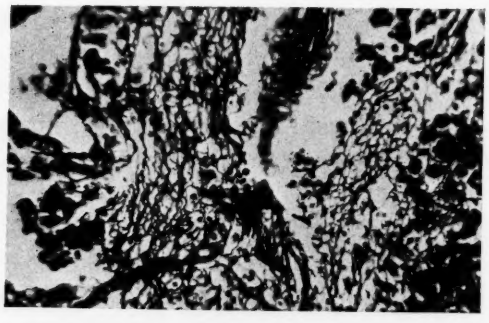

FIG. 22. SECTION IMMEDIATELY FOLLOWING FIGURE 21; THE RETICULUM STAINED. × 200

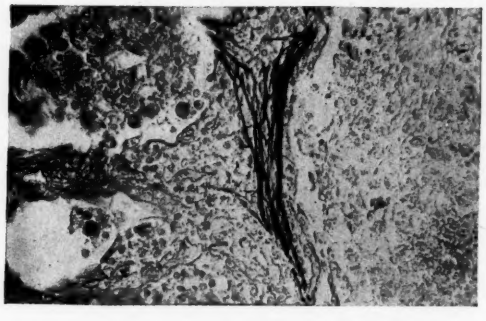

FIG. 23. SECTION IMMEDIATELY FOLLOWING FIGURE 22; THE ELASTIC FIBRES STAINED. × 200

tain a very abundant network which encloses in its mesh the various types of cells (fig. 22). This reticulum is usually continuous with that in the walls of the alveoli, of any division of the bronchial tree, or of any blood or lymph vessel which may be in its immediate neighborhood. Differential staining shows the network of reticulum to be quite distinct from that of the elastic fibres (fig. 23).

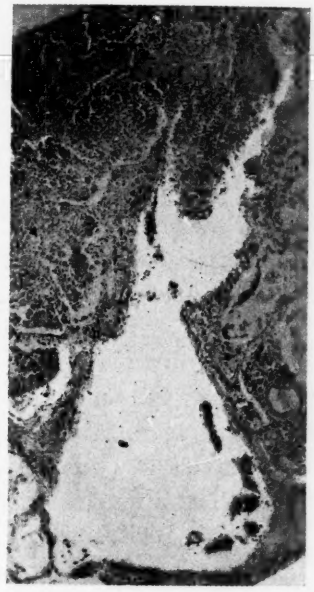

FIG. 24

FIG. 25

FIG. 24. CASEOUS PNEUMONIA

The exudate within the bronchiole is continuous with the exudate within the alveoli. Haematoxylin and eosin staining. × 100

FIG. 25. SECTION IMMEDIATELY FOLLOWING FIGURE 24; THE RETICULUM STAINED

Note that there is *no* new development of reticulum as in figure 22. × 100

Turning now to the second type, an exudative pneumonia which has undergone an early caseation (fig. 24), we see that the pneumonic area outside the bronchiole is becoming caseous at its centre. That it is of recent origin is shown by the pyknotic nuclei immediately outside the caseous centre, while the nuclei around the periphery of the area stain sharply. The wall of the bronchiole adjoining the pneumonic area has

become softened and degenerated, the layer of smooth muscle can no longer be recognized, and the lumen of the bronchiole is filled with a caseating exudate which is directly continuous with the pneumonic area. In the succeeding section (fig. 25) in which the reticulum is stained there is no increase of reticulum in the wall of the bronchiole or of the alveoli.

At the point where the exudate in the bronchiole joins the caseous centre of the alveolar exudate, the reticulum in the wall of the bronchiole is practically destroyed. *No reticulum is present within the alveoli.* This alone differentiates it from the type seen in figures 21 and 22. That differences exist in the character of the exudate in these cases has been previously recognized; that reticulum plays so important a rôle in differentiating the two types from each other has not, so far as the author is aware, been recognized.

We have already seen an example of a tuberculous bronchiolitis (figs. 9 and 10) and have recognized the increased amount of reticulum in and about its wall. We have also seen that wherever the tuberculous process was associated with the formation of giant cells, there was a new formation of reticulum. Figures 26 and 27 are from a series of sections mounted before the method described in the paragraph on technique was adopted. In this series ten consecutive sections were mounted on one slide, the following ten on the next slide, and this was continued until the entire ribbon of sections was mounted. The alternate slides were stained with haematoxylin and eosin, and with Bielschowsky's stain. Because they show a different type of exudate they are introduced in this place. In figure 26 the plane of the section lies through the point where the exudate is continuous with the tuberculous process outside the bronchiole. A careful examination of the exudate within the lumen of the bronchiole discloses that a little to the left and above its centre a small giant cell can be seen. The wall of the bronchiole throughout its entire extent has become involved in the tuberculous process and, along the upper border, disintegrated.

Had the following section been stained for reticulum, it is quite probable that strands of reticulum would have been found extending from the tuberculous mass on the right of the bronchiole into the exudate; for in figure 27 fibres of reticulum can be seen extending between the

epithelial cells lining the interior of the bronchiole, and an occasional fibre is present in the exudate. The reticulum is increased throughout the entire circumference of the bronchiole. Here again we find an increase of reticulum associated with the presence of giant cells.

The histogenesis of reticulum does not properly belong to this contribution. Although the following statement anticipates a future com-

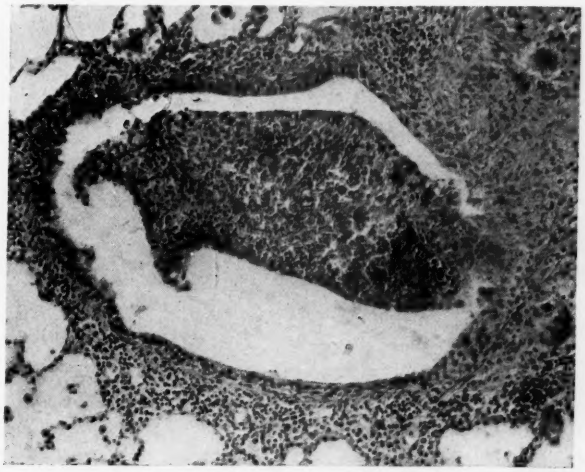

FIG. 26. A PRODUCTIVE TUBERCULOUS EXUDATE WITHIN A BRONCHIOLE. × 75

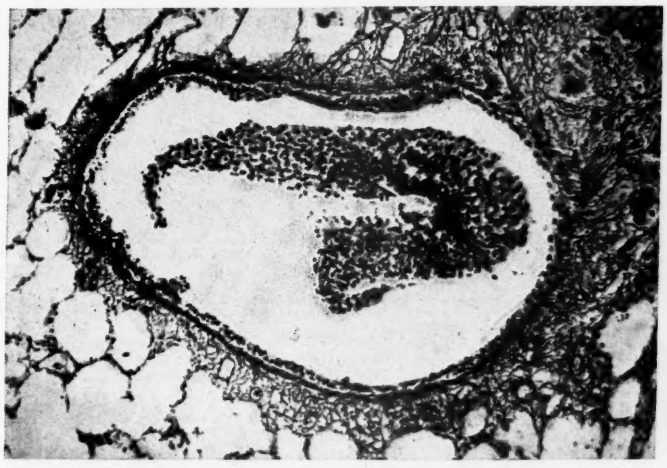

FIG. 27. SECTION THROUGH THE SAME BRONCHIOLE AS FIGURE 26, BUT NINETY MICRA BELOW FIGURE 26; THE RETICULUM STAINED. × 75

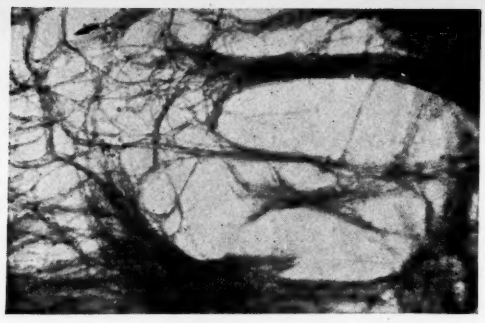

FIG. 28. SECTION THROUGH A CONNECTIVE TISSUE SEPTUM

Pancreatin digestion. Section forty micra thick. Mall's stain. Shows the transition of reticulum fibrils into small strands of collagenous fibres which eventually terminate in well marked bundles of collagenous fibre. × 75

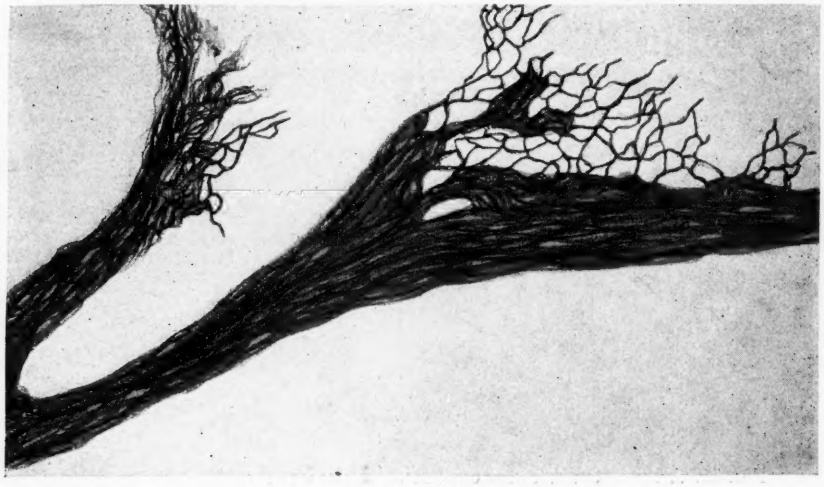

FIG. 29. FROM THE SAME SERIES AS FIGURE 28

Shows the relation of the network of reticulum to a bundle of collagenous fibres. Mall's stain. × 900.

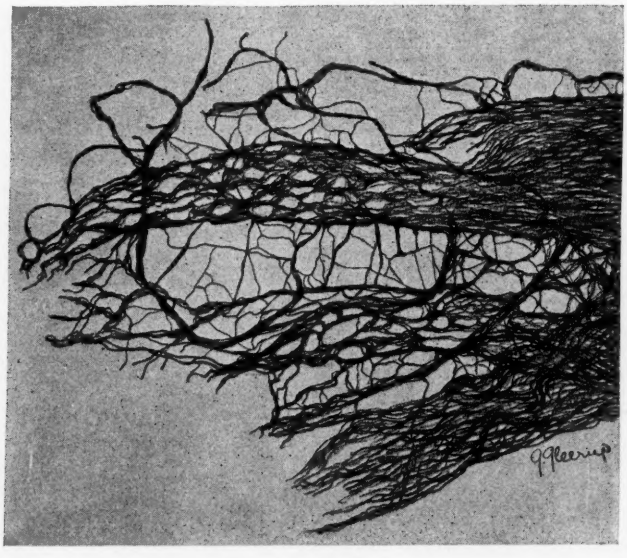

FIG. 30. FROM THE SAME SERIES AS FIGURE 28

Shows the network of reticulum uniting to form sheets and bundles of collagenous fibres. Mall's stain. × 1150.

munication, it may be said that the results thus far obtained from a study of the lung of the child, and of the adult, indicate that reticulum is a precollagenous type of connective tissue, a conclusion previously reached by Russakoff.

If a block of lung, which has been digested *in toto* in pancreatin and cut serially in sections 40μ thick, be stained by Mall's method, it will be found that in the septa between the lobules (secondary lobules) undoubted bundles of collagenous fibres can be seen (fig. 28). If we trace the formation of these bundles it will be found that they originate in a network of very fine branching and anastomosing fibrils—the reticulum. The fibrils of reticulum either unite to form bands of collagenous fibres (fig. 29), or they first form a system of anastomosing bands which eventually fuse into a bundle of collagenous fibres (fig. 30). In this transformation from a network of fine branching and anastomosing fibrils to compact bundles of fibres pressure seems to play an important rôle. This is best illustrated in figure 29. Wagner and some other authors have described reticulum as consisting of a newly formed network of cell processes. This seems hardly possible when one considers the abundant network of fibres shown in figures 28, 29 and 30.

SUMMARY

A giant cell forms the centre of the majority of microscopic tubercles observed in this study.

Coincident with the development of a giant cell newly formed reticulum makes its appearance. This has a tendency to form a basket network of fibres which surrounds the giant cell and encloses within its mesh the epithelioid cells and lymphocytes.

With the formation of a conglomerate tubercle and beginning caseation the reticulum forms a very rich network of fibres *within* and around the caseous centre.

In early caseous pneumonia there is little or no increase in reticulum.

In the case of the so called "lymphoid tubercle" giant cells may be a later development.

This study suggests that, in the case of a tuberculous process associated with the formation of giant cells, there is a more favorable tissue reaction than is the case where, from the first, a caseous pneumonia exists.

Results obtained from this study lead to a provisional statement that reticulum is to be considered a precollagenous type of connective tissue.

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STUDIES ON THE RESPIRATORY ORGANS IN HEALTH AND DISEASE

VIII. A METHOD FOR QUICKLY OBTAINING THE PERCENTAGE OF AN INDIVIDUAL'S THEORETICAL NORMAL VITAL CAPACITY OF THE LUNGS[1]

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In recent years the vital capacity of the lungs of man has been given considerable study by investigators in various parts of the world. It has been proved beyond doubt that the vital capacity test yields information of value as regards the condition of the heart and lungs. When one's normal vital lung capacity has been established by actual observation it is found that disability of clinical significance of the heart or lungs quickly reduces the vital lung capacity. At the present time we have no system whereby vital capacity readings of all normal persons are recorded; therefore, we are forced to resort to theoretical normal standards. Such standards are of necessity more or less unsatisfactory on account of such factors as individual variation. However, they may be reasonably well worked out for people of certain countries and classes. Normal standards have been presented which at the present time are more or less on trial for certain groups of people. True normal standards, however, can be obtained only from observation and analyses of the findings on large numbers of people. Therefore, in this paper an attempt has been made to simplify the vital capacity test, particularly the mathematical calculations, so as to make readily available to all the percentage of the normal vital capacity which a given individual possesses. It is hoped that, with the burden of mathematical calculation lifted, many more physicians and scientists will employ the vital capacity test, and that ultimately any errors in our present normal standards or in our estimation of the value of the vital capacity test will be corrected.

[1]Read in part before the Ninth Session of the Mississippi Valley Conference on Tuberculosis at Columbus, Ohio, September 12–14, 1921.

Prior to 1846 a number of studies had been made upon respiration, but there was wide diversity of opinion as to the amount of air in the lungs at any given time. This diversity of opinion apparently was due to the facts that (1) most workers made observations on a very limited number of individuals, in some instances reporting readings on a single person, (2) little or no consideration was given to the size or condition of the body and (3) there was no standard instrument in use for measuring the air.

In 1846 Hutchinson (1) devised the spirometer. This was a somewhat cumbersome instrument, but was accurate in measuring air. Since Hutchinson's time several different kinds of spirometers have been in use. The water spirometer always gives sufficiently accurate readings. The instrument made by the Sanborn Company, according to the specifications of Peabody and Wentworth (2), is satisfactory in every respect for office and institutional work.

The position of the patient makes a slight difference in vital capacity. A reclining patient may blow as much as 200 cc. less than one in the standing position. When possible, it is best to have all patients in the same position when the readings are taken. Dreyer (3) prefers to have them sitting on a high stool. Most of my readings have been taken with the patient standing. In the case of bed patients, one should add from 100 to 200 cc. to the observed readings.

The patient in the chosen position is instructed to take the deepest possible inspiration; then, in a steady manner, to force all the air possible through the tube of the spirometer. The end of the tube must be inside the mouth, as gross errors may be produced if it is held a short distance from the mouth. The patient should not be allowed to watch the reading scale. Three to five observations are made, and the highest is recorded as the vital lung capacity.

The net weight and standing height (without shoes) of the patient are next recorded. The sitting height is a valuable measurement, as it gives us information as to the length of the trunk and in turn the length of the chest. Dreyer (3) has shown that when the sitting height is taken with the patient sitting on a chair the error may be as great as 3 per cent. Accurate measurements may be taken with the patient sitting on the floor, the knees slightly flexed and the sacrum placed firmly against the door casing. While the patient sits erect the sitting height is taken.

Chest circumference is taken with the chest at rest, but it is difficult to get the patient to hold the chest in this position, the tendency being

to throw out the chest, and hold it in somewhat of a state of expansion. In males the measurement is taken at the level of the nipples. Dreyer (3) has pointed out that in females the chest measurement is best taken below the breasts. Owing to the individual variation in their development, Dreyer has found that the circumference shows an average of 4.5 per cent increase if taken over the breasts. Gray has found that the mean of the chest circumference measurements, taken on forced expiration and the deepest possible inspiration, is an accurate measurement.

From the standing height in centimetres and net weight in kilograms the surface area of the body in square metres is readily obtained from the graphic chart of Du Bois and Du Bois (4). The surface area is said to be one of our most valuable measurements.

The sex and age must always be taken into consideration, since the vital capacity of the female is usually smaller than that of the male, and the vital capacity tends to diminish as the elasticity of the tissues decreases with age.

Normal standards: One of the limitations of the vital capacity test in this country is that the normal standards have not been completely worked out for people of all classes and ages. In 1846 Hutchinson (1) took readings on some 2000 people of England. His subjects were arranged according to occupation and social class, such as policemen, firemen, paupers, sailors, mixed classes, pugilists, pressmen, draymen and gentlemen. Hutchinson used the figures he obtained from these observations as his normal standards when he later attempted to detect the presence of disease of the lungs by means of the spirometer.

Hutchinson's normal standards were used for many decades, but in 1917 Peabody and Wentworth (2) grouped 140 normal persons (96 men and 44 women) into three groups for each sex, according to their standing height. The average vital capacity was taken for each group and the figures used as the normal standard. There are certain obvious objections to a grouping of this kind; however, the standards of Peabody and Wentworth have been used quite extensively. These authors also made a study of the vital capacity in relation to the surface area of the body and found that these two measurements agreed fairly closely. Inasmuch as only a small group of cases was studied in this way, they suggested that observations be made on a larger group.

A number of investigators have reported vital capacity observations in which they have taken the average of a large number of readings.

For example, readings were taken on 1000 young men and the mean vital capacity was found to be 243 cubic inches, while in another instance observations were made on 25,000 young men and the mean vital capacity was found to be 250 cubic inches. It is obvious that averages which do not take into consideration the size of the body cannot be of much practical use. A parallel case would be to take the mean weight of 10,000 men and call this the normal weight for men without taking into consideration such factors as height and age.

Lundsgaard and Van Slyke (5) calculated the chest volume of 18 individuals, including both sexes, and concluded that the vital capacity varies more closely with chest volume than with height.

Dreyer (6) states that the vital capacity test was used quite extensively in selecting men for the British Flying Service. He showed that the minimum requirement was fixed somewhat arbitrarily, and that the size of the individual was disregarded entirely. After making a careful study of this subject, Dreyer concluded that there is a definite relationship between vital capacity and body surface, body weight, stem length and chest circumference. From these measurements he developed mathematical formulae from which one may arrive at the theoretical normal vital capacity of a given person.

In 1920 West (7) published a study, in which he used 85 normal men and 44 women to make a comparison of the various standards for the normal capacity of the lungs. In this work he found that the most accurate method for calculating the normal vital capacity is from a standard based on the surface area. He concluded that the vital capacity should be 2.5 and 2.0 litres of air for each square metre of body surface for men and women, respectively. Due allowance was made for such factors as past physical training.

A study of the vital capacity of 94 normal nurses (8) revealed the mean vital capacity to be 1.87 litres per square metre of body surface. In this work tables were presented showing the vital capacity of normal men and women calculated from one of Dreyer's formulae. It was also shown that two empirical formulae could be used in ascertaining the approximate normal vital capacity for men and women. These formulae were as follows: *17.6 × body weight + 900 = vital capacity for women; 21.2 × body weight + 1168 = vital capacity for men.*

In 1921 Dreyer (3) published a book in which he presented tables showing the calculated normal vital capacities for men and women of different weights, stem lengths and chest circumferences.

Although the tables of Dreyer (3) and the tables made from Dreyer's formulae and the empirical formulae presented by Myers (8) greatly simplified and reduced the work connected with the vital capacity test, there were still time-consuming calculations necessary before one could arrive at the percentage of the normal vital capacity which any individual possessed. The accompanying tables and graphic charts are the result of an attempt to simplify the test by eliminating all mathematical calculations. In producing these tables and charts the mathematical formulae of Dreyer (6), the graphic chart of Du Bois and Du Bois (4), and the observations of West (7) and Myers (8) were used.

In an attempt to test the applicability of the tables calculated from body weight of men, a group of 1280 young men recently studied by Myers and Myers (9) was used. The age of the individuals in this group ranged from seventeen to thirty-two years. Of this entire group, 11.4 per cent had vital capacities below 85 per cent of the normal, while 13.9 per cent had vital capacities above 115 per cent of the normal. The vital capacity readings were taken routinely as the students were examined in the department of physical education of the University of Minnesota. Studies recently completed on more than 1300 college men by Shepard and Myers (10) showed quite similar results.

The tables, calculated both from body weight and sitting height, have been applied also to 400 men of the Minneapolis fire department now being studied by Myers and Maeder (11). Hewlett and Jackson (12), in using some of the formulae from which my tables were derived, found in a study of 400 normal college students that approximately 21 per cent have vital capacities below 90 per cent of the normal, while approximately 2 per cent fall below 75 per cent of the theoretical normal.

Dreyer (6) applied his formula, from which table 1 was made, to 959 Oxford undergraduates studied by Schuster (13), and found the results very satisfactory.

Table 2 was applied to a group of 1058 women, from seventeen to twenty-three years of age, studied by Myers and Myers (14). It was found that 27.1 per cent of the group had vital capacities below 85 per cent of the theoretical normal, while only 3.6 per cent had vital capacities above 115 per cent of the theoretical normal. In this group of women the vital capacity readings were taken as the physical examinations were being made in the department of physical education of the University of Minnesota. The figures in this group are much lower than those obtained by West (7) and Myers (8). From these figures it would appear

that the normal standards are too high for women of the ages represented, or that the test was not properly conducted during the routine examination.

A series of vital capacity readings in the aged, recently completed by Myers and Cady (15), has shown that in most cases the percentages are considerably reduced with age.

Stewart (16), (17), (18), Wilson and Edwards (19), (20), and Emerson and Green (21) have shown that the vital capacity test is valuable in the study of children. They have pointed out, however, that the normal standards used for adults are not applicable for children of all ages.

SUMMARY

1. Numerous studies on the vital capacity test have proved that it renders valuable information as regards the condition of the heart and lungs.

2. The normal standards now in use need to be applied to large series of individuals of different ages, occupations, etc.

3. In the past the mathematical calculations, necessary to arrive at an individual's percentage of the theoretical normal vital capacity, have been quite time-consuming. This fact has undoubtedly discouraged somewhat the application of the vital capacity test to large numbers of individuals both in health and disease.

4. In this paper tables and graphic charts are presented from which one may quickly obtain the percentage of an individual's theoretical normal vital capacity when only a few simple measurements are known. No mathematical calculations are necessary.

I desire to express my thanks to the Hennepin County Tuberculosis Association for financial aid received in the preparation of this paper.

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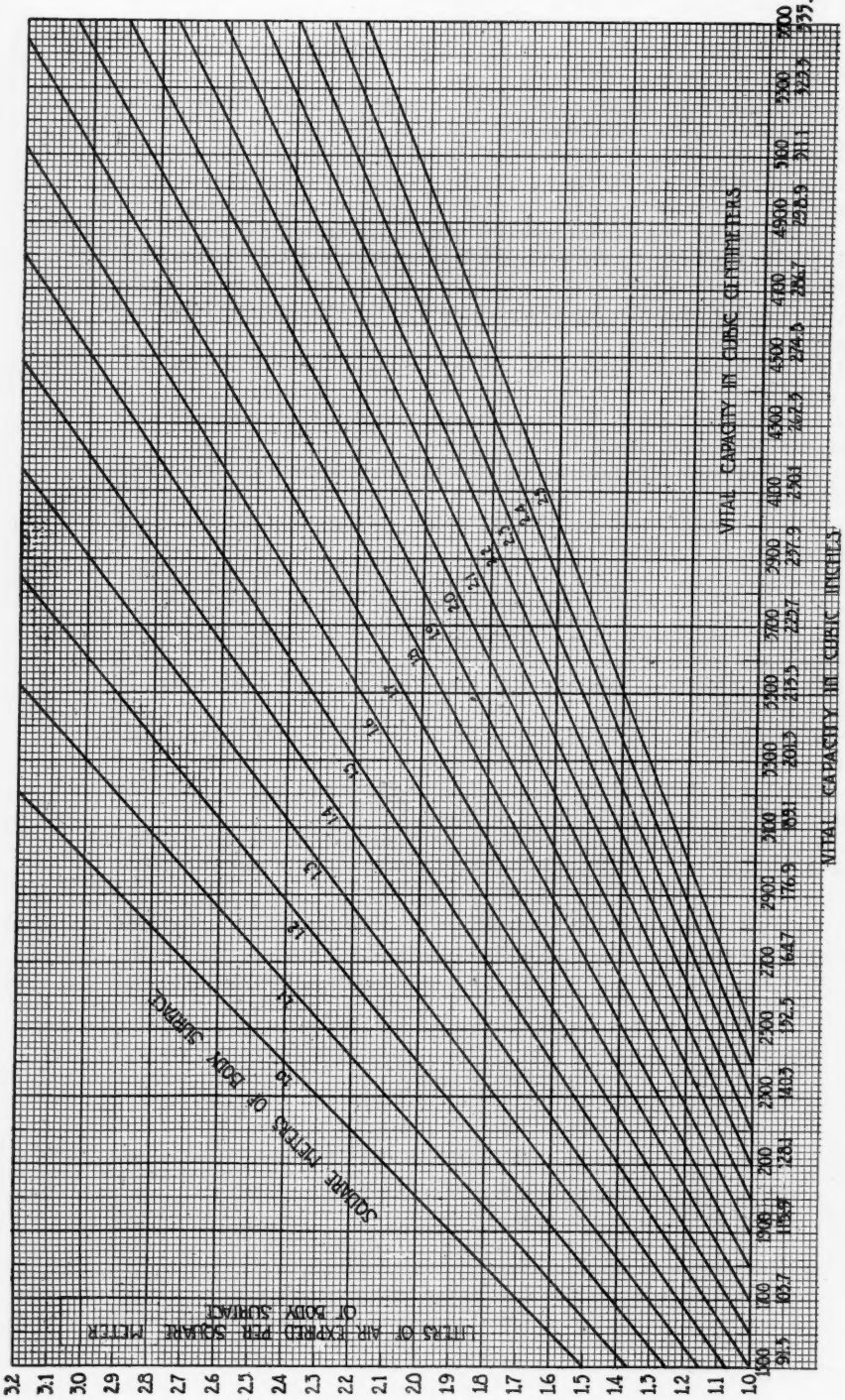
LITRES OF AIR EXPIRED PER SQUARE METER OF BODY SURFACE
SQUARE METRES OF BODY SURFACE
VITAL CAPACITY IN CUBIC CENTIMETERS
VITAL CAPACITY IN CUBIC INCHES

CHART 1: Take the weight in kilograms and the height in centimetres and from the graphic chart of Du Bois and Du Bois obtain the square metres of body surface. With this figure in mind, take the vital capacity of the patient in cubic centimetres or cubic inches. Find in the horizontal column at the bottom of chart 1 the figure corresponding to the observed vital capacity. From this figure follow the vertical line upward to the point where it intersects the diagonal line representing the body surface of the patient. From this point of intersection, follow the horizontal line to the extreme left of the chart, where the figure indicates the litres of air expired per square metre of body surface. For example: A patient has a surface area of 1.5 square metres and a vital capacity of 4500 cc. From 4500 in the horizontal column at the bottom of the chart follow the vertical line upward to its intersection with the diagonal line representing 1.5 square metres of body surface. From this point follow the horizontal line to the extreme left of the chart, where the figure 3.0 indicates the number of litres of air expired per square metre of body surface.

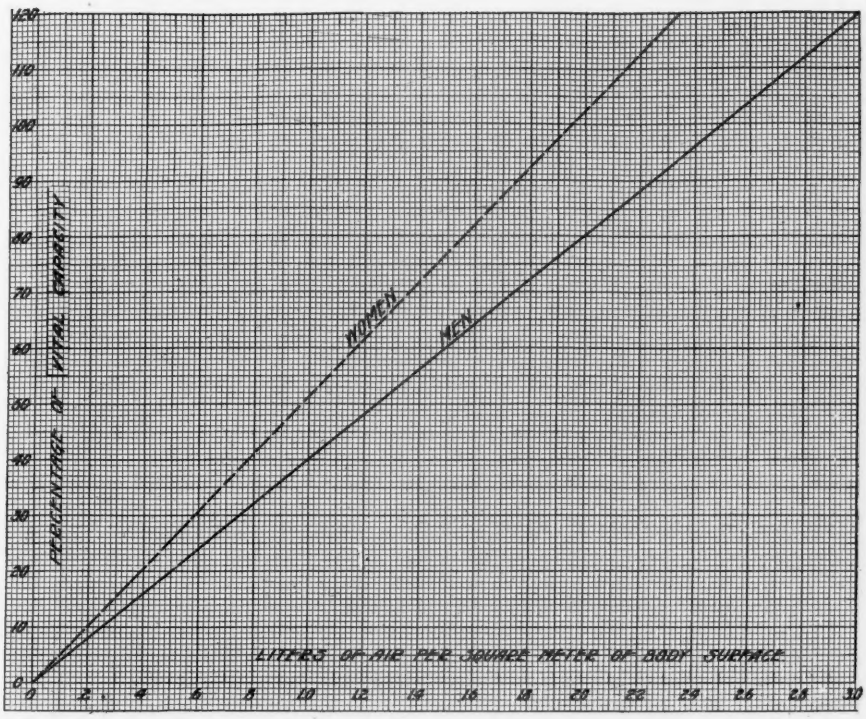

CHART 2: From chart 1 ascertain the number of litres of air the patient expels per square metre of body surface. Locate this figure in the horizontal column at the bottom of chart 2. If, for example, the figure is 2.0, follow the vertical line leading upward from 2.0 to the point where it intersects the diagonal line labelled men. From this point follow the horizontal line to the extreme left of the chart where the figure 80 represents the percentage of the normal vital capacity of the patient. If the patient is a woman one uses the broken diagonal line labelled women instead of the line labelled men.

line upward to its intersection with the diagonal line representing 1.5 square metres of body surface. From this point follow the horizontal line to the extreme left of the chart, where the figure 3.0 indicates the number of litres of air expired per square metre of body surface.

Percentage of Vital Capacity - Men (Calculated from Body-Weight)

| Body weight in pounds | Vital Capacity in Cubic Centimeters | | |

figure is 97 per cent. Corrections may be made in a similar manner when the vital capacity observed falls between the vital capacity figures on the table.)

PERCENTAGE OF VITAL CAPACITY - WOMEN (CALCULATED FROM BODY-WEIGHT)

Body weight in pounds	Vital Capacity in Cubic Centimeters																																															Body weight in kg.
	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	2400	2500	2600	2700	2800	2900	3000	3100	3200	3300	3400	3500	3600	3700	3800	3900	4000	4100	4200	4300	4400	4500	4600	4700	4800	
100	23	26	30	34	38	42	45	49	53	57	60	64	68	72	75	79	83	87	91	94	98	102	106	109	113	117	121																			45.4
105	22	25	29	33	36	40	44	47	51	55	58	62	66	69	73	76	80	84	87	91	95	98	102	106	109	113	116	120																			47.6
110	21	25	27	32	35	39	42	46	49	53	57	60	63	67	70	74	77	81	84	88	92	95	99	102	106	109	112	116	119																		49.9
115	20	23	27	31	34	38	41	44	47	51	55	58	61	65	68	72	75	78	82	85	89	92	96	99	102	106	109	113	116	119																	52.2
120	20	23	26	30	33	36	40	43	46	50	53	56	60	63	66	70	73	76	79	83	86	89	93	96	99	103	106	109	112	116	119																54.4
125	19	22	26	29	32	35	39	42	45	48	51	55	58	61	64	68	71	74	77	80	84	87	90	93	97	100	103	106	109	112	116	119															56.7
130	19	22	25	28	31	34	37	41	44	47	50	53	56	58	62	66	69	72	75	78	81	84	87	91	94	97	100	103	106	109	112	115	119															59.0
135		21	24	27	30	33	36	39	42	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100	103	106	109	112	115	118	121													61.3
140		21	24	27	30	33	35	38	41	44	47	50	53	56	59	62	65	68	71	74	77	80	83	86	89	92	95	98	101	103	106	109	112	115	118	121													63.5
145		20	23	26	29	32	35	38	40	43	46	49	52	55	58	61	64	66	69	72	75	78	81	84	87	90	92	95	98	101	104	107	110	113	115	118	121												65.7
150		20	22	25	28	31	34	37	39	42	45	48	51	53	56	58	62	65	68	70	73	76	79	82	84	87	90	93	96	99	101	104	107	110	112	115	118	121											68.0
155		19	22	25	27	30	33	36	39	41	44	47	50	52	55	58	61	63	66	69	72	74	77	80	83	85	88	91	94	97	99	102	105	107	110	113	115	118	121										70.4
160		19	21	24	27	30	32	35	38	40	43	46	48	51	54	57	59	62	65	67	70	73	75	78	81	83	86	89	91	94	97	99	102	105	108	110	113	115	118	120									72.5
165			21	24	26	29	32	34	37	40	42	45	47	50	53	55	58	61	63	66	68	71	74	76	79	82	84	87	90	92	95	97	100	103	105	108	110	113	116	118	121								74.8
170			21	23	26	28	31	34	36	39	41	44	46	49	52	54	57	59	62	64	67	70	72	75	77	80	82	85	88	90	93	95	98	100	103	106	108	111	113	116	119	122							77.1
175			20	23	25	28	30	33	35	38	40	43	46	48	51	53	56	58	61	63	66	68	71	73	76	78	81	83	86	88	91	94	96	99	101	103	106	109	111	114	117	120							79.4
180			20	22	25	27	30	32	35	37	40	42	44	47	49	52	54	57	59	62	64	67	69	72	74	77	79	82	84	87	89	91	94	96	99	101	104	106	109	111	114	117	120						81.6
	366	427	488	549	610	671	732	793	854	915	976	1037	1098	1159	1220	1281	1342	1403	1464	1525	1586	1647	1708	1769	1830	1891	1952	2013	2074	2135	2196	2257	2318	2379	2440	2501	2562	2623	2684	2745	2806	2867	2928		
	Vital Capacity in Cubic Inches																																															

TABLE 2 is used in the same manner as table 1.

Percentage of Vital Capacity - Men (Calculated from Sitting Height)

| Sitting height in inches | Vital Capacity in Cubic Centimeters | | |

PERCENTAGE OF VITAL CAPACITY WOMEN (CALCULATED FROM SITTING HEIGHT)

Sitting height in inches	Vital Capacity in Cubic Centimeters																																						Sitting height in cm.
	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	2400	2500	2600	2700	2800	2900	3000	3100	3200	3300	3400	3500	3600	3700	3800	3900	4000	4100	4200	4300	4400	4500	4600	
26	22	27	32	36	43	49	54	59	65	70	76	81	86	92	97	103	108	113	119																								66.0
26½	21	26	31	36	41	46	52	57	62	67	72	77	83	88	93	98	103	108	113	119																							67.3
27	20	25	30	35	39	44	49	54	59	64	69	74	79	84	89	94	99	104	108	114	119																							68.6
27½	19	24	28	33	38	43	47	52	57	62	66	71	76	81	85	90	95	100	104	109	114	118																							69.8
28		23	27	32	36	41	45	50	55	59	64	68	73	77	82	86	91	96	100	105	109	114	118																						71.1
28½		22	26	30	35	39	44	48	52	57	61	65	70	74	79	83	87	92	96	100	105	109	113	118																					72.3
29		21	25	29	33	38	42	46	50	54	59	63	67	71	75	80	84	88	92	96	100	105	109	113	117	121																		73.6
29½		20	24	28	32	36	40	44	48	52	56	60	64	68	72	76	80	84	88	93	97	100	104	108	112	116	120																	75.0
30		19	23	27	31	35	39	43	47	50	54	58	62	66	70	74	78	82	85	89	93	97	101	105	108	112	116	120																76.2
30½		19	22	26	30	34	37	41	45	49	52	56	60	63	67	71	75	78	82	86	90	93	97	101	105	108	112	116	119															77.5
31			22	25	29	32	36	40	43	47	50	54	58	61	65	68	72	75	79	83	86	90	94	97	101	105	108	112	115	119														78.7
31½			21	24	28	31	35	38	42	45	49	52	55	59	62	66	69	73	76	80	83	87	90	94	97	100	104	107	111	114	118	121												80.0
32			20	23	27	30	33	37	40	44	47	50	54	57	60	64	67	70	74	77	80	84	87	90	94	97	100	104	107	110	114	117	120											81.3
32½			19	23	26	29	32	35	39	42	45	48	52	55	58	61	65	68	71	74	77	81	84	87	90	94	97	100	103	106	110	113	116	119										82.6
33			19	22	25	28	31	34	37	41	44	47	50	53	56	59	62	65	68	72	75	78	81	84	87	90	94	97	100	103	106	109	112	115	118	121								83.8
33½				21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	100	102	105	108	112	114	117	120							85.1
34				20	23	26	29	32	35	38	41	44	46	49	52	55	58	61	64	67	70	73	76	79	81	84	87	90	93	96	99	102	104	108	110	113	115	119						86.4
34½				20	22	25	28	31	34	37	39	42	45	48	51	54	56	59	62	65	68	70	73	76	79	82	84	87	90	93	96	98	101	104	106	109	112	115	118	121				87.6
35				19	22	24	27	30	33	35	38	41	43	46	49	52	54	57	60	63	65	68	71	73	76	79	82	84	87	90	92	95	98	100	103	106	109	111	114	117	120	122		89
35½					21	24	26	29	32	34	37	40	42	45	47	50	53	55	58	61	63	66	69	71	74	76	79	82	84	87	90	92	95	97	100	103	105	108	111	113	116	118	121		90.2
	814	30.5	36.6	42.7	48.8	54.9	61.0	67.1	73.2	79.3	85.4	91.5	97.6	103.7	109.8	115.9	122.0	128.1	134.2	140.3	146.4	152.5	158.6	164.7	170.8	176.9	183.0	189.1	195.2	201.3	207.4	213.5	219.6	225.7	231.8	237.9	244.0	250.1	256.2	262.3	268.4	274.5	280.6	
	Vital Capacity in Cubic Inches																																									

TABLE 4 is used in the same manner as table 3.

THE DIAGNOSIS OF DOUBTFUL CASES OF TUBERCULOSIS BY EXAMINATION OF BILE OBTAINED THROUGH THE DUODENAL TUBE

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The finding of tubercle bacilli in material from human sources, excluding, of course, the obvious possibility of contamination from outside, is the one incontrovertible piece of evidence establishing a definite diagnosis of tuberculosis. Many cases, though without question clinically tuberculous, fail to reveal the bacillus. There are probably many and intricate factors, pulmonary retention, unconscious swallowing of the sputum, etc., which increase the difficulty of finding the bacilli in the sputum, even though there is distinct clinical evidence of ulceration of the tuberculous foci in the lungs. To place the diagnosis of tuberculosis on a more substantial basis, every possible means of increasing the positive bacillary findings would serve, not only to make diagnosis more certain, but also to exclude more definitely the vague nontuberculous case, and thus aid in making a differential diagnosis.

The method of draining the gall bladder recently advocated by Lyon (1) has placed at our disposal another possible aid in finding tubercle bacilli in the human body. Lyon uses the duodenal tube and the local application of a 25 per cent of a saturated solution of magnesium sulphate as a duodenal douche (Meltzer, 1917). That tubercle bacilli can occur in the bile in certain cases of tuberculosis in man is borne out by bacteriological studies of human bile obtained postmortem by Fraenkel and Krause (2). Of 36 cases of tuberculosis the bile was sterile for rapidly growing microörganisms in 34. The bile from 11 of those found sterile was injected intraperitoneally into guinea pigs, in 1 cc. amounts, and 5 of the injected guinea pigs developed tuberculosis. The "positive" biles were obtained from 2 cases of miliary tuberculosis, one of caseous pneumonia, one of peribronchial tuberculosis and one of progressive pulmonary and intestinal tuberculosis. In none of these cases were any macroscopic changes to be found in the liver. These authors warn against relying

on culture tests for tubercle bacilli in the bile and recommend guinea pig inoculation as the only chance, for as much as 4 cc. of human bile

TABLE 1

Results of the inoculation of guinea pigs with sputum, gastric and duodenal contents, and bile from adult cases of pulmonary tuberculosis, negative for tubercle bacilli by routine repeated smear examination

CASE	NUMBER AND RESULTS OF PREVIOUS ROUTINE SPUTUM EXAMINATIONS DURING HOSPITAL RESIDENCE*	CLINICAL AND ROENTGENOLOGICAL DIAGNOSIS	SPECIMEN INJECTED SUBCUTANEOUSLY INTO GUINEA PIGS				
			Sputum	Gastric contents	Duodenal contents	Bile A	Bile B and C
1	13 times negative	Moderately advanced pulmonary tuberculosis	−	−	−	−	−
2	No sputum; saliva only sent in	Questionable tuberculosis	0	−	−	−	−
3	5 specimens only obtained. No sputum reported	Questionable tuberculosis	0	−	−	−	−
4	18 times negative	Unresolved influenza pneumonia	−	−	−	+	−
5	10 times negative	Questionable pulmonary tuberculosis	−	−	−	−	+
6	Bacilli found once, two years ago, then persistently negative except during an acute cold	Moderately advanced pulmonary tuberculosis	+	†	+	−	+
7	14 times negative	Incipient pulmonary tuberculosis	+	+	+	+	+
8	18 times negative	Moderately advanced pulmonary tuberculosis	+	+	+	+	+
9	8 times negative	Moderately advanced pulmonary tuberculosis	+	+	+	+	+
10	10 times negative	Moderately advanced pulmonary tuberculosis	+	+	+	−	−
11	13 times negative	Moderately advanced pulmonary tuberculosis	+	−	−	+	+
12	16 times negative	Moderately advanced pulmonary tuberculosis	+	+	−	−	+

* The five daily sputum smear examinations prior and subsequent to the guinea pig inoculation tests were negative in all the cases.

† Not obtained.

can be injected intraperitoneally into a guinea pig without harm. They believe that the bacilli originate from the liver, enter the gall bladder with the bile, and are subsequently excreted with the feces.

In 1913 Lydia Rabinowitsch (3), in reviewing the subject of the presence of tubercle bacilli in the circulating blood, mentioned that Maxson found, by staining methods and the antiformin-ligroin technique, acid-fast rods in the bile of 4 of 11 cases with unknown diagnoses. The method is, however, unreliable, as was pointed out by the author. She examined the bile, postmortem, in 17 cases, 16 of which had more or less advanced tuberculosis, while in one case there were only calcified mesenteric lymph nodes. The intestines were macroscopically involved in 11 cases and the liver in 2. Inoculation of guinea pigs with the bile was positive in 12 cases (70 per cent), while microscopically, after antiformin treatment, tubercle bacilli were found only three times. Among these 12 cases intestinal changes were found eight times (once coincident with liver changes). She attributed her high percentage of positive findings to the fact that her cases were more advanced and that inoculations were both subcutaneous and intraperitoneal. In the majority of the cases microscopic examination of the liver revealed no tuberculosis, and she concluded from this that the bacilli reach the liver by way of the blood and are then excreted into the bile, remaining in the gall bladder for a long time without producing changes of any kind. Attempts to cultivate the bacilli from the bile after antiformin treatment resulted negatively in 7 of 8 such studies.

In 1921 Carnot and Libert (4) reported finding tubercle bacilli in duodenal fluid of man. The fluid was obtained by means of a duodenal tube and, though it was claimed that it consisted of bile and pancreatic juice, there was no effort made to stimulate the flow of these secretions and, if they were present, they apparently were found in normal amounts. In all 6 cases of advanced pulmonary tuberculosis, tubercle bacilli were demonstrated in the duodenal fluid, by direct smear of a centrifugated specimen. To determine the value of the examination of the "duodeno-pancreatic" fluid for diagnostic purposes, 7 patients were examined, 3 being positive, and these are reported in detail as follows: 1. A case of tuberculous peritonitis without pulmonary signs gave a positive finding upon direct smear of the "duodeno-pancreatic" fluid. A subsequent examination by direct smear was negative, but a guinea pig (the only one inoculated in the study) was positive forty days after injection. 2. A case of adenitis in which the "bile" was positive. 3. A case of peritonitis with high fever, in which the "duodeno-pancreatic" fluid was negative, even after administration of tuberculin; but after the administration of 30 grams of sodium sulphate by mouth on the

next day a positive test was obtained. A group of 11 nontuberculous cases were negative throughout. The authors concluded that, since the three cases in which tubercle bacilli were found in the duodenal fluid were "closed cases" (by smear only and not by animal inoculation), the tubercle bacilli found in the duodenal fluid were of biliary origin. No tests of the duodenal fluid were made for the presence of bile and no proof was advanced that duodenal fluid was really obtained; in spite of this it was stated that tubercle bacilli were eliminated in the bile and pancreatic juice even when there was no expectoration.

In view of the frequent occurrence of cases of almost indisputable clinical pulmonary tuberculosis which, however, fail to reveal bacilli in the sputum even after careful guinea pig inoculation or after repeated microscopic examination of smears, and the possibilities offered in examining the bile as is indicated by the above review, it seemed desirable that such a study be undertaken; separating, however, as far as possible, the material examined into its various constituents, such as sputum, gastric contents, duodenal contents and bile (the latter obtained by means of the duodenal tube and a 25 per cent of a saturated solution of magnesium sulphate douche according to Meltzer and Lyon).

Two groups of subjects were thus studied; a set of 12 adult patients with distinct pulmonary findings and repeated negative sputum reports, and a set of children, inmates of an orphans' home, where a group of 7 children had been exposed to infection from a fatal open case of pulmonary tuberculosis in a child.

TECHNIQUE FOR OBTAINING THE SPECIMEN FOR THE DETERMINATION OF THE PRESENCE OF TUBERCLE BACILLI

Sputum was collected in a thoroughly clean bottle and examined microscopically (carbol-fuchsin smears), on five consecutive days before and five consecutive days after the collection of the specimen used for guinea pig inoculation and of the gastric, duodenal and bile secretions. All the guinea pigs were injected subcutaneously in the groin. Gastric, duodenal and bile secretions were collected through a sterile duodenal tube. Immediately before passing the duodenal tube, the posterior pharyngeal wall and soft palate were anesthetized by applying *anesthesine* in powder form; this procedure proved invaluable in tuberculous patients, in whom the introduction of the duodenal tube caused spasmodic cough and excited a copious expectoration and even vomiting. All samples were collected in the morning before any food had been taken, to avoid any possible contamination from this source. As the tube

reached the stomach a sample of gastric fluid was withdrawn. When the tube reached the duodenum (this being determined fluoroscopically in the early part of the work and later by the reaction of the fluid and the ability to recover only a few cubic centimetres of water several minutes after injection) a sample of duodenal fluid was carefully withdrawn. Then 35 to 45 cc. of a 25 per cent of a saturated solution of magnesium sulphate of 37°C. was introduced into the duodenum through the duodenal tube. When bile began to appear it was withdrawn in two fractions; the first light bile *A*, and the darker *B* and lighter *C* (Lyon) in the second fraction. The bile was not aspirated, but was siphoned into the receiving vessel. The whole procedure required about one to two hours.

The five specimens (sputum, gastric contents, duodenal contents, bile *A*, and bile *B* and *C*) were treated with 3 per cent sodium hydroxide for twenty minutes (Petroff's method), centrifugated, decanted, the sediment washed with sterile 0.9 per cent sodium chloride solution, and finally injected subcutaneously into five separate guinea pigs. The guinea pigs were kept under careful observation for three months, after which they were sectioned and examined for the presence of tuberculosis. Direct smears stained with carbolfuchsin were examined from all the specimens obtained with negative results in all cases.

Control tests: In order to control the action of the various fluids, mixed with magnesium sulphate, upon the tubercle bacillus, the following control tests were made:

To the samples of sputum, gastric contents, duodenal contents and bile samples *A*, and *B* and *C*, obtained after the use of 40 to 50 cc. of 25 per cent of a saturated solution of magnesium sulphate from two subjects, there was added to each a few drops of a suspension of virulent human tubercle bacilli (0.1 mgm. per cc.), and after twenty-four hours (a longer interval than was required in any of the experiments reported, between the time of collection of the specimen and injection into the guinea pig) the sample was treated with 3 per cent sodium hydroxide for twenty minutes at incubator temperature, and after centrifugation and thorough washing of the sediment with sterile saline solution it was inoculated into guinea pigs subcutaneously. All of the animals developed a marked tuberculosis within three to four weeks after inoculation, indicating that the above procedure was in no wise detrimental to the viability of the human tubercle bacilli.

Adults studied: A total of 12 adult patients of the National Jewish Hospital for Consumptives was examined, 3 of them questionable cases of pulmonary tuberculosis and the remainder with negative sputum but clinically active tuberculosis cases. Their histories follow:

1: P. K., male, 39 years of age, with disease of ten years' duration, recorded clinically as moderately advanced pulmonary tuberculosis, with roentgenological shadows in both apices and considerable fibrosis in the right lung. Glandular and dense infiltrations in both hilum regions. History of bronchiectasis and pulmonary hemorrhages five years ago, with an occasional moderate rise in temperature and moderate cough and expectoration. Sputum examinations persistently negative for tubercle bacilli, at monthly intervals, for six months prior to this study and thereafter. All specimens of sputum, gastric contents, duodenal contents and two bile specimens negative by guinea pig inoculation.

2: S. J., male, 19 years of age, disease of three years' duration; clinical diagnosis questionable tuberculosis; roentgenological examination revealed both apices slightly cloudy. No elevation of temperature but slight cough and absence of expectoration. Sputum examinations repeatedly marked "no specimens" and "saliva only." All specimens at time of test, including bile, proved negative after guinea pig inoculation.

3: M. M., male, 15 years of age, disease of 3 years' duration. Clinical diagnosis, cervical adenitis, with moist râles constantly present in the upper left lung; roentgenological examination of the chest negative. Cough and occasional rise in temperature present. Repeated monthly sputum examinations for one year prior to study negative. All specimens, including bile, proved negative in guinea pig inoculation tests.

4: M. B., male, 21 years of age, and duration of disease about one and a half years. Tentative clinical diagnosis, unresolved influenzal pneumonia, with possible bronchiectasis; roentgenological findings, peribronchial thickening with dense mottling in the lower lobe of the left lung. Occasional rise in temperature, lasting a few days, with marked cough and mucopurulent sputum. Repeated monthly sputum examinations for one year prior to study were negative for tubercle bacilli. At time of test sputum, gastric and duodenal contents and bile *B* and *C* were negative by guinea pig inoculation, while the guinea pig that received bile *A* was markedly involved when examined three months after inoculation, indicating the presence of viable bacilli in this specimen of bile.

5: A. S., male, 33 years of age and duration of disease about one and a half years. Clinical diagnosis, questionable pulmonary tuberculosis; roentgenological findings, slight apical increase in density in upper lobe of right lung with slight peribronchial thickening throughout both lungs. No elevation of temperature or cough and no appreciable sputum. Claims that tubercle

bacilli were found in the sputum six months prior to test, but repeated sputum examinations during the four months under observation were negative. Patient appears robust. At the time of test sputum, gastric and duodenal contents and bile *A* were negative by guinea pig inoculation, while bile *B* and *C* produced a mild generalized tuberculosis in a guinea pig examined three months after inoculation.

6: M. S., male, 18 years of age, duration of disease about two years. Clinical diagnosis, moderately advanced pulmonary tuberculosis; roentgenological examination of the chest revealed considerable infiltration of the entire right lung and slight infiltration of the upper lobe of the left lung. Occasional rise in temperature, with cough and expectoration at times. Sputum examinations were positive once at the beginning of illness two years ago and then became negative by smear until eight months ago, when, during a period of a mild cold, they again became positive upon monthly examination but have been persistently negative since then and until tests were made. During the test the five daily smears prior to and subsequent to the test were negative. At the time of test the sputum (gastric contents not obtained), duodenal contents, and bile *B* and *C* all produced a generalized tuberculosis in the guinea pigs examined three months after inoculation, while the guinea pig receiving bile *A* was negative at that time.

7: J. M., female, 20 years of age, with duration of disease about six months. Clinical diagnosis, incipient pulmonary tuberculosis; roentgenological examination revealed inflammatory mottling in both upper lobes. There were frequent rises in temperature, with cough and expectoration present. The sputum was examined microscopically by stained smears fourteen times during residence in the hospital and was always reported negative. The five smear examinations prior and subsequent to the test were negative. At the time of the test the sputum, gastric and duodenal contents, bile *A* and bile *B* and *C* all produced a generalized tuberculosis in the guinea pigs examined two and a half months after inoculation.

8: H. B., male, 36 years of age, with duration of disease about three years. Clinical diagnosis, moderately advanced pulmonary tuberculosis with emphysema; roentgenological examination revealed infiltration and inflammatory mottling in the upper lobe of the right lung and at the hilum on both sides. There was cough and expectoration but no rise of temperature. The laboratory records were negative for tubercle bacilli in the sputum in spite of 18 examinations being made. The five smears examined microscopically prior and subsequent to the test were negative. At the time of the test the sputum, gastric and duodenal contents, bile *A* and bile *B* and *C* all produced a general-

ized tuberculosis in the guinea pigs examined two and a half months after inoculation.

9: H. G., male, 21 years of age, duration of disease about one year. Clinical diagnosis, moderately advanced pulmonary tuberculosis; roentgenological examination revealed inflammatory mottling in the left apex, with dense infiltrations throughout the entire left lung and slight infiltration in the right apex. Hemoptysis occurred at the beginning of the disease about one year ago. Loss of weight, cough, expectoration and marked dyspnea were recorded. Elevation of temperature was absent. Repeated examinations of smears (eight times) during the four months' residence in the hospital were negative. The five smears examined microscopically prior and subsequent to the test were negative. At the time of the test the sputum, gastric and duodenal contents, bile *A* and bile *B* and *C* all produced generalized tuberculosis in the guinea pigs examined three months after inoculations.

10: M. L., male, 22 years of age with duration of disease about 6 months. Clinical diagnosis, moderately advanced pulmonary tuberculosis; roentgenological findings were inflammatory mottling and infiltration of the apex of the left lung. There were marked loss of weight, moderate cough and absence of fever. Repeated examinations of the sputum, ten times during four months' residence in the hospital, were negative. The five smears of sputum collected five days prior and subsequent to the test were negative microscopically. At the time of the test the sputum, gastric and duodenal contents produced generalized tuberculosis in guinea pigs examined two and a half months after inoculation, while bile *A* and bile *B* and *C* guinea pigs, examined three months after inoculation, revealed no tuberculosis.

11: P. S., male 20 years of age, with duration of disease about one year. Clinical diagnosis, moderately advanced pulmonary tuberculosis; roentgenological examination revealed peribronchial infiltration and thickening, extending to both apices and to the periphery in both lungs, with marked infiltration on both sides at the hilum. Cough and expectoration moderate, and during hospital residence there were several abrupt rises in temperature. During a period of six months in the hospital the sputum had been examined by smear thirteen times, with negative findings for tubercle bacilli. The smears examined daily five days prior and subsequent to the test were also negative. At the time of the test the sputum, bile *A*, and bile *B* and *C*, inoculated into guinea pigs, produced generalized tuberculosis in these animals three and a half months later; while the gastric and duodenal contents failed to infect the animals.

12: C. D., male, 36 years of age, with disease of about two years' standing. Clinical diagnosis, moderately advanced pulmonary tuberculosis; roentgenological examination revealed inflammatory mottling of both apices, with infiltration throughout the left lung. There were moderate cough and expectoration, but no elevation of temperature recorded during residence in the hospital. Entrance records showed that bacilli had been found in 1921. Repeated sputum smear examinations (sixteen times) were negative. The daily specimens examined five days prior and subsequent to the test were also negative. At the time of the test the sputum, gastric contents and bile *B* and *C* produced generalized tuberculosis three months after inoculation, while the duodenal contents and bile *A* failed to infect the guinea pigs.

The above 12 adult cases group themselves into several classes: three (*1*, *2* and *3*) very doubtful tuberculosis cases which were negative for guinea pigs; two cases (*4* and *5*) with positive findings only from the bile; four cases in which practically all inoculations resulted positively; one case (*10*) in which the stomach and duodenal contents were positive while the bile was negative; and two cases (*11* and *12*) with positive results in several specimens, which indicated the importance of testing by inoculation all the separate specimens.

It seems from these studies that, when repeated negative sputum reports are obtained in suspected or questionable cases of pulmonary tuberculosis, the sputum, gastric and duodenal contents, and bile *A* and bile *B* and *C*, obtained according to the method described, should be inoculated separately into guinea pigs for the purpose of demonstrating the presence of tubercle bacilli in these fluids. The reports in the literature and our observations suggest that bacilli may at times be found in the bile when the sputum and gastric and duodenal contents are negative. Our experiments would also seem to indicate that there may be two independent sources from which the bacilli may be obtained; either from the lung, when they may be found in the sputum or gastric or duodenal contents, or from the liver, when they may be found in the bile or duodenal contents. There is also the possibility of any of these fluids being positive without the others.

Studies on children: The following study on children is reported here merely in brief. It will be given in more detail in a subsequent paper which will include a larger number of cases under study. It emphasizes the importance of the study of the bile by guinea pig inoculation in suspected cases, and suggests an important method for the examination

of children for tuberculosis, in whom it is often difficult to obtain sputum specimens and the tendency of the disease is toward rapid and fulminating systemic development, which may possibly increase the opportunity for finding the bacilli in the bile.

The early diagnosis of tuberculosis in infants is often difficult, since symptoms, such as cough and expectoration, are usually lacking, while physical signs are lacking in 80 per cent of the cases even though the roentgen ray may reveal considerable involvement. The Pirquet is of only slight value, since 8 per cent of infants under one year of age may give a positive reaction and when a considerable tuberculous involvement exists this test may be negative. It is important to discover the tuberculosis case in the child early, not only from the standpoint of treatment, but especially, as the following report shows, from the standpoint of contagion, when the child is associated with other young children, as might occur in an orphanage or in large families.

This report concerns 8 children of an orphanage where contact was fairly intimate.

One contagious child was an infant one year old, who entered the orphanage with no obtainable family history, was undernourished and appeared sick. It never gained weight but had frequent attacks of "colds." High temperature developed seven months after admission and the physical examination was recorded as negative. The child was then isolated and died two weeks later, when autopsy revealed a tuberculous bronchopneumonia with miliary dissemination. No Pirquet test had been made.

A second contagious child, one year and five months old, was admitted to the same ward as the first, just two months after admission of the latter. Its family history was negative and it was well nourished. Five months after admission it began to suffer from frequent "colds" and lost weight; the Pirquet test was negative at ten months; and eleven months after admission it began to have fever as high as 104°F. and never lower than 101°. Physical examination of the chest at this time was negative, while the roentgenological examination revealed a bilateral tuberculous bronchopneumonia, miliary in type. It died one month later. No autopsy was obtained.

Pirquet tests were made on all the children in the same ward with the contagious cases, ten months after admission of the second, and 16 of the 27 gave a positive test. The usual positive percentage for this orphanage during the past two years has been 4 per cent. Seven of the

strongly reacting children and a 7 months' old normal child (negative Pirquet) were included in the series from whom bile was obtained through an ordinary duodenal tube (Jutt), in the morning before breakfast or the partaking of any food. A gastric specimen was obtained and, when the duodenal tube passed into the duodenum, 8 to 12 cc. of a 25 per cent saturated solution of magnesium sulphate at 37°C. was introduced, and the bile, appearing in about ten to fifteen minutes, was collected by siphonage. The mixed bile, without fractionation, was used for inoculation into guinea pigs. The entire procedure required from forty-five minutes to one and a quarter hours. The specimens, gastric contents and bile, were treated with an equal volume of 3 per cent sodium hydroxide for 20 minutes at 37°C., washed with saline solution and the washed sediment injected subcutaneously in the groins of guinea pigs. All the specimens proved negative for tuberculosis except one, when the guinea pigs were examined 3 months after inoculation. The positive specimen was bile obtained from a child about two years old at the time of test.

This child had a negative family history and had been healthy, with none of the diseases of childhood. The Pirquet test was positive in the tenth month after admission of the second contagious child to the orphanage and again three months later. Physical examination revealed enlarged cervical lymph nodes on the right side, with a slight decrease in resonance of the right chest. The roentgenograph revealed a fan-shaped infiltration, extending from the hilum to the periphery in the upper lobe of the right lung, with enlarged hilum nodes. Only one of the other 7 cases (ranging from seven months to four years) presented, clinically or roentgenologically, any evidence of even slight tuberculous involvement, and this case showed only suspicious pulmonary findings, with slightly enlarged hilum nodes roentgenologically.

Here we have, then, in a series of 7 contact cases, one with definitely positive bile. Careful clinical and roentgenological study has confirmed this as a case with pulmonary involvement. It emphasizes the importance of the examination of the bile, by this method, in establishing a definite diagnosis and it would seem that the harmlessness of the procedure would recommend it, at least in doubtful cases and in differential diagnosis. It would be well, also, to call attention to a possible source of contamination, such as the presence of bovine tubercle bacilli in unpasteurized milk, against which precautions should necessarily be

taken. Where bovine bacilli are found (and it is recommended that in all cases a differential test should be made) the test might be considered as of practically no value as compared to the finding of human bacilli.

SUMMARY

In questionable or doubtful cases of pulmonary tuberculosis, in which routine sputum smears have failed persistently to reveal tubercle bacilli, it is recommended that guinea pig inoculation be resorted to, using for this purpose sputum and gastric and duodenal contents and bile obtained by means of the duodenal tube and a duodenal douche of magnesium sulphate solution (Meltzer and Lyon).

In a series of 12 such cases in adults there were 2 in which the bile alone was capable of infecting guinea pigs, while the sputum and gastric and duodenal contents were negative. In the case of 7 children in an orphanage, exposed to two fatal cases of severe pulmonary tuberculosis, tubercle bacilli were found in the bile of one of the contacts by this method, thus clarifying and corroborating the clinical and roentgenological findings in these cases. Where the possibility of milk contamination exists this should be guarded against by testing the strain of bacillus found, preferably by rabbit inoculation.

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SOME REMARKS ON THE DIFFERENTIAL DIAGNOSIS OF TUBERCULOSIS VERSUS HYPERTHYROIDISM[1]

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The close resemblance of tuberculosis and hyperthyroidism has occasioned great difficulty in their differentiation. A history of early fatigue, weakness, palpitation, feverishness and nervousness might suggest either of the two conditions. In the absence of other symptoms and definite clinical findings the diagnosis would remain in doubt. Such a hypothetical situation is not rare in practice. The symptoms are often indefinite or confusing. A patient may complain of pleurisy pains and cough, suggesting tuberculosis, together with such thyroid symptoms as extreme nervousness, choking sensations and palpitation. The physical or clinical findings may seem to conflict, as when a patient has exophthalmos and lung infiltration. Similarly, the symptoms may not fit in with the clinical findings, or vice versa. A variety of combinations is possible, and often occurs, in which some feature of the case is incongruous with a diagnosis of either tuberculosis or hyperthyroidism. This accounts for the difficulty of differentiating these two conditions.

In 1918 Goetsch (1) described a test of epinephrin hypersensitiveness which, if positive, is generally supposed to indicate hyperthyroidism. This has since been used extensively as a differential sign. However, its value is strictly limited, as it is not pathognomonic. Heise and Brown (2) later found that 14 per cent of tuberculosis cases were hypersensitive to epinephrin.

There is still another consideration in estimating the value of the Goetsch test. Theoretically, it is possible that tuberculosis coexists with hyperthyroidism. If so, it is unjustifiable to rule out tuberculosis simply because the patient is hypersensitive to epinephrin. More will be said of this subsequently.

Another differential test is the metabolism rate. Authorities state that hyperthyroidism is accompanied by increased metabolism. There

[1] Read before the Kalamazoo Academy of Medicine, September 26, 1922.

is still some doubt in my mind as to how far this holds true. In my experience many patients with signs and symptoms of hyperthyroidism have shown a normal metabolic rate. In other words, I believe that slight hyperthyroidism may occur without measurable change in metabolism. Such a discrepancy might be partially explained by the assumption that metabolism rate is subject to comparatively rapid changes, whereas the physical findings are relatively more constant.

If, then, the epinephrin test and the metabolic rate do not serve to differentiate the borderline case, we must rely on some other factor to make a diagnosis. Such a factor is presented herewith, in the hope that it will simplify the diagnosis of tuberculosis and hyperthyroidism.

It is not unreasonable to assume that these two conditions may occur together. Such a coincidence would explain the apparent incongruity of symptoms or signs which has offered so much difficulty heretofore.

A study of 52 consecutive cases of pulmonary tuberculosis suggests that hyperthyroidism is a frequent complication or sequel of the disease. Eighteen of these cases showed definite thyroid symptoms and three others showed doubtful findings.

The following is a typical case report:

Mrs. E. L. C., a housewife of thirty-three years, came in with a presumptive diagnosis of "ulcer of the bowel." Two months before she had been forced to stay in bed because of cramping pain in the abdomen and fever, which reached 104°. The fever subsided fairly promptly and in a month she left her bed. However, she continued to lose weight. Her previous weight was 125 pounds but at examination she weighed 94 pounds. She had practically no other symptoms except weakness and some pain in the right chest. The physical examination showed old pleuritic adhesions at the right base and active tuberculosis in the left upper lobe, with typical posttussic râles. This was in 1921, about fifteen months ago. The patient was placed on rest treatment and she improved rapidly, gaining 21 pounds. She returned to work and felt well for several months.

Last January she developed an ulceration of one of her teeth and this was followed by a return of the pains in the stomach and a laryngeal irritation, which caused a persistent nonproductive cough. The temperature was found to be 102° each afternoon. The lung examination showed active involvement of the right upper lobe, as well as some râles over the old lesion on the left. A provisional diagnosis was also made of tuberculosis of the larynx. The patient was extremely nervous and irritable; even the rustling of a newspaper could not be endured. She slept very poorly. The signs of hyperthyroidism became more manifest as the nervousness increased and the temperature

reached 103° or 104°. The thyroid gland was palpated only with difficulty and has shown no swelling or change in consistency. No bruit was present over the gland. The pupils were large and equal and reacted normally to light and accommodation. The Stellwag sign was present. The palms were warm and moist. The pulse was fast and out of proportion to the temperature. The skin was warm and the patient required very little covering even in cool weather. There was a fine tremor of the fingers. The knee and elbow reflexes were exaggerated very markedly. The metabolism was plus 4 per cent. The thyroid symptoms gradually subsided until the temperature was practically normal and riding in a wheel chair was well borne. Certain slight signs have remained, such as tachycardia, exaggerated reflexes, slight nervousness and a modified Stellwag sign. However, the pulmonary tuberculosis is progressive.

This case shows a chronic tuberculosis with a thyroid phase superimposed for a time. The tuberculosis seems to be the initial lesion and the thyroid disturbance is probably secondary. In a sense the hyperthyroidism is a complication of the tuberculosis. However, it may be worthy of remark that this patient resembles the thyroid type. She has always been active and highstrung. She has always been slender although her parents and her only sister are well-nourished. Her appearance suggests that in her earlier life she may have had a previous attack of tuberculosis with recovery, but her history gives no indication of such an occurrence.

It is interesting to speculate on the cause of the thyroid symptoms in this case. A tuberculous invasion of the thyroid gland is possible. Plummer (3) has reported seven cases of tubercle in the thyroid, most often associated with pulmonary and glandular involvements. Earlier reports are given by Ruppanner (4) and by Werdt (5). Fraenkel (6) has proved that in generalized miliary tuberculosis the thyroid is practically always involved.

The short duration of the thyroid phase in this case would not accord with the chronicity of a tuberculous lesion. Nor does it seem probable that a tuberculous invasion could destroy enough thyroid tissue in so brief a time as to explain the disappearance of the hyperthyroid symptoms.

The rareness of thyroid tuberculosis also speaks against such a diagnosis. Mosiman (7) found less than 1 per cent of tuberculosis in a series of thyroids removed in Crile's clinic.

On the other hand, thyroid complications in tuberculosis of the lungs are probably common. In our series of 52 cases there were 18 with hyperthyroid symptoms. The incidence was highest among patients in the quiescent stage.

There are but few references in medical literature to this subject. Tice (8) speaks of symmetrical thyroid enlargement in early tuberculosis and mentions the presence of gland tenderness in advanced tuberculosis. Fishberg (9) found that enlargement of the gland is not rare in youthful tuberculous patients and that mild exophthalmos is not uncommon. Norris and Landis (10) found that out of 2212 patients at the Phipps Institute in Philadelphia one out of sixteen showed thyroid enlargement, many of these with eye signs.

From these statistics it seems justifiable to assume that thyroid enlargement and hyperthyroidism may occur in the course of tuberculosis. Pottenger (11) undertakes to explain the interrelationship of the two conditions. He believes that the toxemia of tuberculosis stimulates the sympathetic nervous system; that sympathetic stimulation increases the flow of adrenin; that increased flow of adrenin calls for increased flow of thyroid secretion; that increased function of the thyroid gland is accompanied by increased flow of blood and increase in the size of the cellular components of the gland, thus resulting in hyperplasia of the thyroid. Webb (12) has shown that, experimentally, tuberculous guinea pigs develop hyperplasia of the thyroid. Pottenger further states that these changes are temporary and that the gland returns to normal after the toxemia disappears. If we accept this reasoning we have an explanation for the clinical fact.

Now, to return to the question of differentiation between tuberculosis and hyperthyroidism, shall we not confess that this problem has lost much of its difficulty? The presence of confusing symptoms is easily explained on the basis of the coincidence of the two diseases. The presence of thyroid symptoms and signs need not overthrow our diagnosis of tuberculosis, as the two are not incongruous.

Another case report will illustrate how this theory aids in reaching a diagnosis.

Mr. C. W., aged thirty-one years. This man was first examined about two years ago and diagnosed as a case of pulmonary tuberculosis, moderately advanced and active. He complained of weakness, fever, pain in the chest, cough and expectoration. The physical examination showed an afternoon

rise in temperature, emaciation, flat chest with retraction of the right apex, percussion dulness at both apices, exaggerated whispered voice and breath sounds at the right upper, and posttussic râles in the second intercostal space.

Eighteen months later his examination shows a flat chest, dull at the right apex, with a narrow Kronig's isthmus. The other lung sounds are normal. The skin is slightly pale. The patient is still underweight. The thyroid gland is barely palpable and normal in consistence. The Stellwag sign is positive. The palms are moist and warm. There is a slight tremor of the fingers. The tendon reflexes are increased. The blood pressure is 132 systolic and 70 diastolic, readings which are high for an asthenic patient. The hemoglobin is 85 per cent. The red and white blood cell counts are normal. The temperature is usually normal but occasionally it goes to 99.5°. The pulse varies from 80 to 100. The metabolism is plus 4 per cent.

The patient states that he is not well. Some days he feels quite well but at other times he feels badly. He can do very little work and tires quickly. He is very restless, but gets rid of this feeling sometimes by taking a brisk walk. He frequently has a heavy feeling in the region of the heart.

Here is a patient with apparently arrested tuberculosis. The signs of tuberculosis are only slight and do not account for his continued poor health. The tuberculosis has cleared up but a thyroid disturbance is present.

Compare this picture with the ordinary case of quiescent tuberculosis. The latter looks well and feels well and altogether is happy and optimistic. This man is almost the opposite. In short, the typical case of tuberculosis is benefited by a sanatorium regimen and shows favorable results, while the other shows improvement in his physical findings but symptomatically remains unwell.

As regards the differential diagnosis, this case would offer little difficulty in the early stage when the evidence clearly indicated tuberculosis. If the patient were first seen at the time of the second examination a thyroid disturbance would have been suggested.

This patient is typical of many borderline cases in which it is so difficult to rule out either tuberculosis or hyperthyroidism. Yet the explanation seems simple if we assume that the hyperthyroidism was present as a complication or sequel of the tuberculosis.

That a number of our patients had both tuberculosis and hyperthyroidism seems fairly well established. The diagnoses of tuberculosis were based on positive sputum, posttussic râles, definite fibrosis, hemoptysis and pleural effusion. The thyroid symptoms and signs were less

definite. Most of the patients showed nervousness, tachycardia, tremor of the fingers, moist warm palms, exaggerated reflexes, retraction of the upper eyelid, mild emaciation, and neurocirculatory asthenia. The symptoms consisted of inability to work hard, "easy tire," sudden changes from a state of feeling well to an "all in" feeling, restlessness, irritability, insomnia, sweating, feverishness, chilliness, numbness, palpitation and choking sensations. Not all of these signs and symptoms occur in the same patient. The usual picture shows an asthenic type, nervous and underweight. The eyes show no proptosis, but the upper lid retracts occasionally while the patient is conversing or focusing the eyes. The thyroid is normal on examination or shows a slight uniform enlargement. The heart shows poor muscular tone and marked tachycardia after exercise or deep breathing. The palms are usually moist and warm. The biceps, triceps and prepatellar reflexes are often very prompt and exaggerated. The blood pressure may be low for both systolic and diastolic readings, but not infrequently both are comparatively high for the general condition of the patient. The metabolism is usually normal but may be markedly increased.

The patients in this series have ranged in age from thirteen to thirty-five years, with a single patient of forty-five years. There has been no noticeable preponderance of either sex.

The majority of patients showed a quiescent stage of tuberculosis. In several cases the tuberculosis was present before the hyperthyroid symptoms developed. In no case was a tuberculous lesion seen to follow hyperthyroidism.

In most of our cases there was no appreciable enlargement of the thyroid, but a small percentage showed slight uniform hypertrophy without change in consistency. If Pottenger's theory of hyperplasia is correct, there is presumably some enlargement of the gland although it might be so slight as to escape detection clinically. A true hyperplasia probably occurs when the tuberculous infection runs a prolonged course of activity. However, where the active stage is of short duration, it is conceivable that hyperthyroidism may result without hyperplasia as the latter would develop rather slowly. Similarly, in a progressive tuberculosis, we are likely to find the hyperthyroid symptoms present long before the hyperplasia becomes manifest. Therefore the absence of clinical enlargement of the thyroid in early tuberculosis does not militate against Pottenger's theory.

In arrested tuberculosis, on the other hand, it seems that hyperplasia is necessary to explain the persistence of hyperthyroid symptoms. In the absence of tubercle-toxin absorption we would expect that the thyroid would return to normal function unless it had undergone a permanent change, such as hyperplasia. The series of cases which form the basis of this discussion is far too short to warrant any conclusions as regards this question, except to show that the thyroid manifestations do persist after the tuberculosis becomes arrested. However, it is possible that certain thyroid manifestations are permanent even though the glandular hypersecretion disappears.

CONCLUSIONS

Pulmonary tuberculosis is frequently complicated by hyperthyroidism.

Clinical evidence indicates that hyperthyroidism is secondary to the tuberculosis.

A true thyroid hyperplasia probably results in some cases from the toxemia of tuberculosis.

The theory of coincident tuberculosis and hyperthyroidism explains the difficulty hitherto experienced in differentiating these two conditions.

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THE ALKALI RESERVE IN TUBERCULOSIS

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Modern blood chemistry has played a great part in clearing up unknown factors of disease. Among others, the tests for the establishment of the H-ion concentration of body fluids have contributed materially to that end. Such determinations as of the plasma bicarbonate of Van Slyke (1), of the carbon dioxide content and capacity of the whole blood and plasma of Henderson (2), of the alveolar carbon dioxide of Friderica (3) and Haldane (4), and the various hydrogen ion determinations of the blood are a few familiar examples.

There have been found various conditions where the body "acidity" varies from the normal. For example, diabetes, nephritis with retention, diarrheas in infants, etc., have all been found either to increase the acidity or lower the alkali reserve (or both) in the body fluids.

In consideration of the above mentioned fact it seemed desirable to study the alkali reserve in a series of cases of pulmonary tuberculosis, inasmuch as in this disease one of the chief organs of elimination is so materially affected. It is not, however, the scope of this paper to enter into a discussion of the theory of acidosis of related principles.

During part of one year, therefore, we made a study of the blood chemistry of 488 cases of pulmonary tuberculosis in the Municipal Tuberculosis Sanitarium of the City of Chicago, with the object of ascertaining the change of alkali reserve during the different stages of the disease. Of this number only 262 cases are actually tabulated in the main series. The other cases were omitted because many of them were children with lymph node tuberculosis; and many were too old or too young to fit into the scheme of analysis hereinafter outlined.

Because of its simplicity, the Van Slyke method for the determination of bicarbonate of venous blood plasma was used. As will be shown later, this method is satisfactory in most cases and will show all that is necessary in the problem under consideration. A subsequent work will follow wherein arterial blood will be used and absorption curves plotted.

EXPERIMENTAL

The blood was drawn without stasis from the patients within a week after they had entered the institution. The determinations were performed within three days (usually the same day) from the date the blood was drawn. All samples that were not free from hemoglobin were rejected, as were all cases that were not classified within two weeks from the time the blood was drawn. Three modifications of Van Slyke technique were used that had for their objects the economy of time and material rather than greater accuracy, although the accuracy was in no way vitiated. First the plasmas were resaturated with carbon dioxide in a 250 cc. Erlenmeyer flask instead of the usual separatory funnels, after a doubly perforated cork, delivery tubes and pinchcocks were supplied.

Another change in the technique consisted in making an emulsion of 0.02 cc. caprylic alcohol in 1.5 cc. of 5 per cent sulphuric acid before using. After the air bubbles were dispersed from this emulsion, 1 cc. was pipetted into the receptacle at the top of the apparatus and lowered into the chamber as usual. This was done because the caprylic alcohol is very difficult to get into the chamber without wasting considerable quantities of it, using too much, or running the risk of letting in air. When too much is used, the mercury becomes emulsified and must be cleaned more frequently. A third variation was in the method of exhausting the gas. The apparatus was not removed from the rack to "shake out" the gas. Instead, the left hand grasped the apparatus at the level of the lower two-way stopcock and the thumb held the stopcock firmly. Then the right hand gradually lowered the mercury reservoir until the liquid was below the stopcock mentioned, when the movement was quickly reversed, thus forcing the liquid through the stopcock like the spray of an atomizer. After repeating this five times, there would appear no bubbling of the liquid, indicating that exhaustion of the gas was complete. This fact was confirmed by actual tests because no increase in reading was obtained by further exhaustion. The purpose of this change in technique was to abolish the risk of dropping the apparatus when it was removed from the clamps. Stadie (5) had this same object in view when he recently devised his shaking apparatus as an attachment.

The calculations were made from Van Slyke tables after the volume had been corrected for barometric pressure.

Table 1 represents the findings in 262 cases of uncomplicated tuberculosis. The patients include incipient, moderately advanced and far advanced cases, and range in age from sixteen to fifty years. Patients younger than sixteen were not included because those under that age normally have a lower alkali reserve than adults. Those over fifty were excluded for three reasons: (1) there were only a few cases, (2) there is a tendency in old people to develop acidosis and (3) there were several "fibroids" in this group.

TABLE 1

	AGE 16 TO 20		AGE 21 TO 25		AGE 26 TO 30		AGE 31 TO 35		AGE 35 TO 40		AGE 40 TO 50		TOTAL NUMBER OF CASES	AVERAGE CO_2 READING
	Number of cases	CO_2 reading	Number of cases	CO_2 reading	Number of cases	CO_2 reading	Number of cases	CO_2 reading	Number of cases	CO_2 reading	Number of cases	CO_2 reading		
Incipient:														
Male	4	53.0	7	54.3	11	52.9	4	55.1	3	57.6	4	57.4	33	54.4
Female	11	51.7	9	49.4	8	51.4	9	48.5	6	52.8	6	54.8	49	51.1
Moderately advanced:														
Male	6	52.3	7	53.8	11	53.1	7	53.0	6	52.9	7	52.9	44	53.0
Female	8	50.8	11	50.0	11	50.3	6	50.5	6	50.8	5	52.0	49	50.6
Far advanced:														
Male	3	51.9	5	49.6	12	51.5	6	51.5	6	51.6	2	53.9	34	51.4
Female	8	50.0	10	50.7	7	47.1	15	50.0	12	52.2	3	49.7	55	50.2
Average:														
Male		52.4		52.3		52.5		52.9		53.2		54.5		
Female		50.9		50.1		49.8		49.6		52.0		52.5		

The 262 tabulated cases were arranged according to age, sex and stage of the disease. Only three stages were considered; incipient, moderately and far advanced. It will be noted that there is a difference of two to three volumes per cent between the male and the female patients, that there is a very slight lowering of the alkali reserve as the disease progresses, and that in this series there is a marked lowering of the alkali reserve between the ages of twenty-five and thirty-five in the females. The curve for men does not show a corresponding depression of the alkali reserve at this age period.

Chart 1 shows a curve that has been plotted to illustrate some of these points. The chart also shows the results obtained on 123 children.

The majority of these children were lymph node cases, although some of them were undernourished children who were under observation and a few may have been normal.

At this point it may be well to state that after one hundred tests had been made the results were so inconclusive that abandonment of the work was considered. However, the work was continued in the hope that a grand average of several hundred patients would reveal something of value. The hope has been but partially realized. There is a slight depression of alkali reserve in a grand average of tuberculous patients, as compared to a grand average of normal individuals who are in good health and on an average diet. In no stage of the disease was there

CHART 1

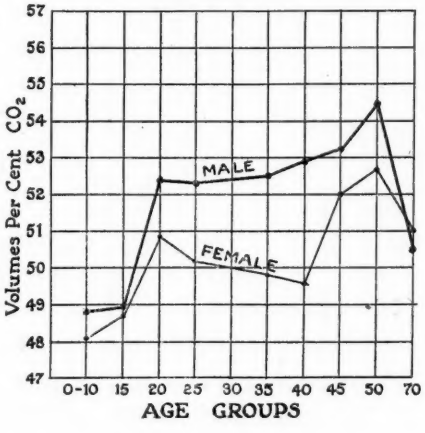
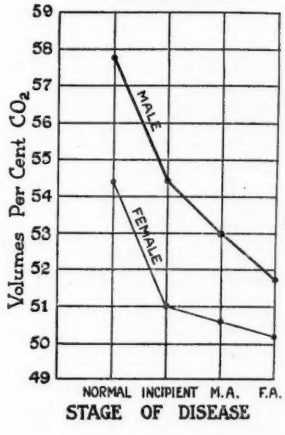


CHART 2



found an abnormally low alkali reserve or a true "acidosis" as a result of tuberculosis alone.

The different stages of the disease are plotted in chart 2. The females showed a smaller decrease in alkali reserve than the males, that is, the curve is almost flat, but the cases as a whole were lower in proportion than the male cases.

Nine normal men and 4 normal women were selected as controls for the whole series. The average for the normal men was 57.8 and for the normal women 54.2. From the tuberculous women 15 "fibroids" were selected, and the average for them was 52.1 volumes per cent which is considerably higher than the general average for the tuberculous women. The objection may be raised quite properly that the control series is too small. The reason for this is that the author has had the

privilege of performing a rather large number of tests on individuals apparently in good health. The results of these 13 control examinations coincided so well with the results obtained on this earlier series, and with the averges of other workers, that a larger series was deemed unnecessary.

DISCUSSION

In a large series of tuberculosis patients there seems to be a very slight depression of alkali reserve which becomes slightly increased as the disease progresses. It must be pointed out, however, that individual cases may not conform to the curves, which have been plotted; for example, some early cases of tuberculosis gave low readings, lower, in fact, than could be explained by tuberculous involvement alone. On the other hand, there were a few patients who were classed as "far advanced C" and who subsequently died, who gave readings higher than the average normal. The whole problem must be interpreted in the light of our knowledge of lung volume in tuberculosis, "minute volume" in tuberculosis, alveolar and arterial carbon dioxide tension, H-ion concentration, carbon dioxide content of arterial and venous blood, the effect of oxygen unsaturation and hemoglobin content of the blood.

Whether this slight depression is caused by the pulmonary involvement or is due to the general effect of the disease on the cells of the body is a question. It seems reasonable that tuberculous involvement would play a rôle in the alveolar gas exchange. In moderately or far advanced tuberculosis, where the vital capacity and lung volume are decreased, the parenchyma of a large part of the lung is clogged with caseous masses, the bronchioles are plugged with mucus and adjoining tissue is inflamed in such a manner that there are good reasons to believe that the function of these alveoli is greatly impaired. The surface will eventually be reduced to such a point that, when all alveoli work to capacity, gas will not pass through with normal speed and oxygen will diffuse more slowly than carbon dioxide, so that a progressive anoxemia will result. As a consequence, the respiration is increased to supply the oxygen, and the carbon dioxide is "washed out" in the process, resulting in a reduced alkali reserve, increased minute volume and decreased alveolar carbon dioxide tension. On the other hand, there is no proof against the theory that it is due to a simple accumulation of acid from increased metabolism and impaired cell function. The work of Lundsgaard and Van Slyke (6) on vital capacity, lung volume and residual air in tuber-

culosis furnishes valuable information in this connection. They showed that lung volume and vital capacity was markedly decreased in late tuberculosis.

Before any further discussion it will be advantageous to summarize Van Slyke's work, and then we shall be able to explain certain facts more clearly. Van Slyke (7) has made use of the work of Haggard and Henderson, of Peters, Barr and Rule and of his own, and has charted the possible conditions found in human blood. He divided the whole field into nine groups, plotted by allowing the two extreme normal pH lines to cross the two extreme absorption curves, thus dividing the field into nine parts. There are high, medium and low pH zones, each of which has a high medium and low carbon dioxide content. Henderson has a similar classification but his is not so detailed. At any rate, there are several distinct groups. Van Slyke calls the first an unsaturated alkali excess, produced by overdosage of alkali, or too much washing of the stomach, thus taking out too much acid. This condition is characterized by a slow respiration, due to the lowering of the H_2CO_3—$NaHCO_3$ ratio, an increase in alveolar carbon dioxide, an increase of bicarbonate in the urine and a decrease of ammonia excretion. The symptoms are almost identical with those of tetany. Then there is the uncompensated carbon dioxide deficit, when H_2CO_3 has been eliminated more rapidly than $NaHCO_3$. It is caused by hyperpnea, either voluntary or by breathing air with reduced oxygen content. The clinical symptoms and laboratory findings are similar to those mentioned in the first class, except that they are not so severe.

Another condition is that in which both the H_2CO_3 and the $NaHCO_3$ are high, but properly proportioned; hence there is a normal pH. There may be a compensated alkali excess following administration of alkali, when the absorption has been rather slow, so that the H_2CO_3 could keep pace with $NaHCO_3$. There is also a compensated carbon dioxide excess that exists in emphysema, in which the retarded gas exchange increases the carbon dioxide in the blood. This type may be differentiated from the previous one by the fact that cyanosis accompanies it, due to a slower diffusion of oxygen than carbon dioxide, as shown by Krogh (8). When the carbon dioxide is hindered, oxygen is retarded still more, so that cyanosis is the result. There is the normal bicarbonate content compensated with carbonic acid. This varies for each individual, but lies roughly between a carbon dioxide tension of 25 to 63 volumes per cent, a carbon dioxide content of 35 to 63 volumes per cent and a pH of

7.30 to 7.45. Another class is that in which both the H_2CO_3 and $NaHCO_3$ are low, but properly balanced. Like the conditions in which both are high, it may be due to a deficit of one or the other. For example, the compensated alkali deficit may be caused by an increase of nonvolatile acids in the blood. The carbon dioxide keeps pace and maintains a constant pH. This is no doubt the most important class of all. In diseases such as diabetes, nephritis, marasmus, etc., nonvolatile acids occur that reduce that alkali reserve and hence fall into this group. In the other type the carbon dioxide is lowered by rapid breathing or by a stimulation of the respiratory centre with oxygen deficit, such as happens in mountain climbing or in reduced oxygen from any cause.

Still another type is an uncompensated carbon dioxide excess, in which there is a retention of carbon dioxide without any compensation of $NaHCO_3$. This occurs when the respiratory centre is deadened or by breathing air that is surcharged with carbon dioxide. It is characterized by an increased elimination of ammonia and acids in the urine and an increase in minute volume in the lungs, in an apparent attempt to rid the body of carbon dioxide.

The last condition is that in which the bicarbonate reserve is reduced beyond the point of compensation and the H_2CO_3—$NaHCO_3$ ratio increases, as well as the H-ion concentration. This is known as uncompensated acidosis.

We may examine these various conditions to see which ones may be found in tuberculosis, and, if found, to see which ones may be identified by the technique used. In the first place, no information is gained regarding the H-ion concentration from the plasma bicarbonate determination. A high bicarbonate content may have a low, normal or high pH, but the pH and bicarbonate are high together only after alkali administration, or when acid is taken from the body. A normal pH with a high bicarbonate must have a compensated carbon dioxide excess, or compensated alkali excess. The latter appears usually after moderate dosage of alkali, or, theoretically, after a moderate reduction of hydrochloric acid from the stomach. Either of these conditions is hardly probable in tuberculosis. As to the compensated carbon dioxide excess, similar to the type found in emphysema, such a condition may be possible in tuberculosis. The alveoli may be damaged so that the diffusion of gases is slower than normal. If both oxygen and carbon dioxide are influenced as in emphysema, there will be a cyanosis which sometimes exists to a slight degree in tuberculosis. This condition may account for some of the high readings found in far advanced cases.

In the groups in which the bicarbonate is normal, if the pH is high, it became so by hyperpnea, caused either by a reduction of oxygen tension such as changing to a higher altitude or by excessive breathing. It is a fact that excessive breathing occurs in tuberculosis. The minute volume is increased, as shown by McCann (9). However, if the breathing is increased, the bicarbonate content will soon decrease and with it the pH. As the bicarbonate decreases to the point where the H_2CO_3—Na HCO_3 ratio is normal, the pH will accompany it. It is more probable that in the reduction of the alkali reserve, the process is so slow that the pH does not rise above normal high enough to measure, but the bicarbonate decreases very gradually and keeps the pH normal.

Passing to the normal bicarbonate with a low pH, which is caused by a retention of carbon dioxide, we have a condition that cannot possibly be identified by the analysis of plasma bicarbonate. There is no way of telling this form from a normal one. If there is a retention of carbon dioxide due to a hypothetical deadening of the respiration centre, an increase in minute volume and an increase in acid and ammonia excretion in the urine will accompany it. Besides, the condition will tend to pass into the group having a high bicarbonate content, which, as pointed out before, can easily be identified. The symptoms alone will be the determining factor in these cases.

The condition in which the bicarbonate is low and the pH is high, as shown before, cannot remain long, if it ever does occur, in uncomplicated tuberculosis cases in normal surroundings. At the other extreme, where the pH is low and the bicarbonate is low, there is never any difficulty in placing these cases because the symptoms of uncompensated acidosis render the diagnosis easy.

Therefore, the types are few and unimportant that cannot positively be placed after a careful study of the symptoms and the venous plasma bicarbonate determination. For the general purpose of this paper the technique used has answered the purpose. A more complete study is now being made, however, involving the absorption curves of arterial blood, with a careful check on symptoms and acid and ammonia in the urine.

CONCLUSION

Although these results may be considered negative as far as proving that tuberculosis is accompanied by an "acidosis," yet there is an indication that the general average of tuberculosis patients have a slight

tendency in that direction and that the careful administration of alkalies, either in the form of food or drugs, will remove one barrier, though slight, from the way of the convalescent patient. Too much protein, therefore, from which amino and other acids are derived would be contraindicated. The administration of acetates, citrates or sodium bicarbonate would be beneficial. A diet with the maximum amount of vegetables and with a limited amount of meats would be preferable to a heavy meat diet. This is confirmed by Lusk (10) who states that H-ion concentration is greater on a meat than on a vegetable diet.

SUMMARY

1. The plasma bicarbonate determination of Van Slyke was performed on 262 specimens of plasma from adults having uncomplicated tuberculosis and on 126 specimens of plasma from children.

2. Tuberculosis alone is not necessarily accompanied by a true "acidosis."

3. An average of a large series of tuberculosis patients, although still well within normal limits, has a decreased alkali reserve 4 to 5 volumes per cent below a grand average of individuals in apparently good health.

4. Female patients have, uniformly, an alkali reserve that is 2 to 3 volumes per cent lower than that of male patients, while children and old people have an alkali reserve lower than both the former.

5. Fibroid cases have a higher average of alkali reserve than patients having the same amount of involvement more acutely.

6. Certain far advanced cases gave relatively high readings.

The author wishes to express his appreciation to Dr. John Dill Robertson, President of the Board of Directors, whose efforts have made this work possible, and to Dr. Allan J. Hruby for his coöperation.

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THE X-RAY IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS[1]

A COMPARISON OF X-RAY AND PHYSICAL SIGNS IN 1000 CASES

HARRY LEE BARNES

Wallum Lake, Rhode Island

The material for this study consists of 1111 patients at the State Sanatorium at Wallum Lake, Rhode Island. The cases are consecutive in so far as there are complete clinical and X-ray records. A considerable number of doubtful cases of lung disease, sent to the Sanatorium for diagnosis but not admitted, have been added. Although nearly all cases were believed or suspected to have pulmonary tuberculosis, the diagnosis of tuberculosis of the lungs, including suspects, was confirmed in only 1000 of the 1111 patients.

The great majority of the physical examinations were made by the writer, and the X-ray interpretations represent the composite views of the Sanatorium medical staff, in which the writer shared.

Although the fluoroscope is frequently used by the staff in pneumothorax and other work, it is not used for the diagnosis of doubtful cases and we have had little experience in its use for such purposes. Stereographic films were made routinely in negative sputum cases.

In order that the physical examinations and film readings may be absolutely unbiased, one should be ignorant of the X-ray findings when examining the chest and ignorant of the identity of the patient whose film is being read. The personal equation and judgment enter very largely into the detection and interpretation of X-ray shadows, just as they do in the detection and interpretation of physical signs, and yet we must rely largely on this comparison of physical and X-ray findings in forming our opinion of what changes have taken place in the lungs.

POSITIVE SPUTUM CASES

As the X-ray examination is much more expensive than a sputum examination, where economy is urgent the X-ray can be reserved for

[1] Read at the New England Conference on Tuberculosis, Augusta, Maine, September 28, 1922.

the negative sputum cases. Our diagnosis should, however, be accurate enough to tell us whether or not both lungs are involved and to what degree; and the value of the X-ray is so great that all institutions for tuberculosis should take at least one flat picture in all positive sputum cases. Of the 592 positive sputum cases in this series, the X-ray showed an involvement of one apex which had not previously been revealed by physical signs in 39 patients, or 6.5 per cent. This percentage would be considerably increased had slight dulness, which is a doubtful sign, been eliminated.

Of these 592 positive sputum patients, only 5 were read as negative to the X-ray, and reëxamination of the films showed that in 4 slight but definite X-ray evidence had been overlooked at the original interpretation, and in the remaining patient tubercle bacilli were reported in the sputum before admission, but were not found by us. With rare exceptions, a negative X-ray in positive sputum cases means poor technique or inaccurate interpretation of the plates. Where suspicious physical signs are found at an apex and the X-ray is negative, it is best to restudy the plate, and when the X-ray shows densities in areas previously found free from physical signs it is wise to reëxamine the lungs. Where the physical signs are few and doubtful, and with good technique the X-ray is negative, in the absence of convincing symptoms of tuberculosis one feels justified in delaying the diagnosis.

NEGATIVE SPUTUM CASES

Of the series of 519 negative sputum cases, in only 9 were both apices clear and the X-ray evidence of tuberculosis found elsewhere. It must then be chiefly in the apices that we are to find the changes in density which will help us in the diagnosis of the doubtful cases. Our findings in 519 negative sputum patients are shown in table 1.

From the above table it will be seen that in 64 per cent of negative sputum cases, physical signs at the apices were accompanied by X-ray signs over the same area. When the râles are few and faint and the dulness doubtful, it is a great comfort to have X-ray confirmation of the accuracy of the physical examination.

It is an interesting question as to how frequently the X-ray fails to show evidence of a recent tuberculous process. Of 592 positive sputum cases, only 11 or 2 per cent had one or more apices with positive physical signs and negative X-ray findings, while, taking the lungs as a whole,

in 36 or 6 per cent the areas having abnormal physical signs exceeded the areas having abnormal X-ray densities. The X-ray can hardly be expected to show the slightly increased secretion in the bronchi which would produce râles. The number of positive sputum cases where, over areas showing râles the X-ray findings are for a time negative but later positive, indicating a primary failure of the X-ray to find the lesion, is small, probably not more than 2 per cent.

TABLE 1

Analysis of X-ray densities with physical signs

NUMBER OF APICES	PHYSICAL SIGNS	X-RAY FINDINGS AT APICES POSITIVE IN 64 PER CENT					
		Specks	Streaks	Haziness	Cloudiness	Mottling	Negative
728	Dulness or moist râles present.....	9 or 1 %	44 or 6 %	74 or 10 %	202 or 28 %	139 or 19 %	260 or 36 %
310	No dulness or moist râles.....	1	17 or 5 %	46 or 15 %	50 or 16 %	38 or 12 %	158 or 51 %
1038		10 or 1 %	61 or 6 %	120 or 12 %	252 or 24 %	177 or 17 %	418 or 40 %

TABLE 2

Analysis of physical signs with X-ray densities in negative sputum cases

NUMBER OF APICES	X-RAY	PHYSICAL SIGNS			
		No signs	Dulness only	Râles only	Dulness and râles
10	Specks.....	1 or 10 %	5 or 50 %	0 or 0 %	4 or 40 %
60	Streaks.....	17 or 28 %	17 or 28 %	3 or 5 %	24 or 39 %
120	Hazy.....	46 or 38 %	30 or 25 %	8 or 7 %	36 or 30 %
190		64 or 34 %	52 or 27 %	11 or 5 %	64 or 34 %

It will be noted in table 1 that where the physical signs were negative the X-ray signs were also negative in 51 per cent and positive in 49 per cent. Marked dulness is of course unmistakable, but marked dulness at one apex leaves us no standard by which to judge the second apex. Where dulness is very slight the writer believes himself to be in error in the technique of percussion or its interpretation in from 10 to 20 per cent of the cases, and many physicians of special experience in lung

examination have confessed to me their own uncertainty in relying on percussion where the impairment of the percussion note is slight. Much of the error of percussion can be eliminated by reëxaminations.

In apices in which the X-ray shows marked clouding or opacity, we usually find consolidation postmortem. If the consolidation is unevenly distributed or contains small cavities the X-ray may show mottling.

With good technique, haziness at the apex, even though slight, usually means abnormality of lungs or pleura. Some apices with marked dulness and râles show only haziness. Three plates recently seen with hazy apices showed postmortem, two of them in less than a month afterward, dense adhesions about the apex. One apex, in which rarefaction was seen through a thick haze, showed postmortem the apex almost completely excavated and encased in an unusually thick tough pleura. From the last case it appears probable that a thick pleura alone can cause a hazy apex. A haze sometimes remains after the absorption of tubercles from lung tissue.

Small specks on the bronchi are not always tuberculous (Dunham), and we dare not say that a fine speck or two anywhere is certainly abnormal. Several sharply defined specks from the size of a pinhead to the size of a shot (28 in distance) in the apex, are usually conglomerate tubercles in many stages of activity, fibrosis and calcification, but we must also admit that they may be sometimes, if rarely, nontuberculous in origin. They are often not very active, as judged by the absence of râles. In a recent case in which the X-ray reading at the left apex was "a few fine specks," the necropsy report on the left apex three days later was "moderate amount of infiltration with conglomerate tubercles."

Peribronchial streaking, if generally distributed throughout both lungs, is frequently a result of a nontuberculous process, but if more prominent at one or both apices it suggests tuberculosis. Where several heavy peribronchial lines enter the apex, there is usually slight dulness, even though no general haziness or mottling can be made out.

Although the difference in density shown by X-ray will not tell us from what infection the patient suffers, it will in many cases show the morbid changes of the lung better than any method of physical examination. Scientific interpretation of very slight X-ray changes in the apices, seen on the best films, must await necropsy findings, and as patients with these signs usually continue to live it may be a long wait. The sudden death by accident of some of these patients, previously X-rayed, may eventually give us the material.

In diagnosing negative sputum patients suspected of tuberculosis, one seeks by physical and X-ray examination to find the evidence of condensation of lung tissue which will fit in with the history of past and present symptoms, and by careful balancing of probabilities give us a diagnosis which, though lacking the accuracy obtainable by necropsy, is nevertheless the best that can be done for the living patient.

SUMMARY

1. Of 592 positive sputum patients only 5 were read as negative to the X-ray and rereading of the films showed that 4 of the 5 had slight changes at the apex.

2. Of 592 positive sputum cases, X-ray evidence of involvement of the second apex, unrevealed by physical examination, was shown in 6 per cent.

3. Of 592 positive sputum patients, in 380 or 64 per cent, the X-ray evidence of disease was more extensive than the physical signs; in 36 or 6 per cent, the physical signs were more extensive; and in 176 or 30 per cent the amount of lung involvement was about the same as indicated by the two methods.

4. Of 310 apices of negative sputum patients, in which dulness or moist râles had not been found, 40 per cent showed abnormal X-ray densities.

5. Of 728 apices of negative sputum patients, showing dulness or moist râles, 36 per cent were negative to the X-ray.

6. In negative sputum patients both dulness and râles were found in 40 per cent of the apices showing specks, 39 per cent of apices showing streaks and 30 per cent of apices showing haziness.

THE SUBSEQUENT HISTORY OF CHILDREN DISCHARGED FROM TUBERCULOSIS SANATORIA[1]

ARTHUR T. LAIRD

Nopeming Sanatorium, Nopeming, Minnesota

Considerable emphasis is being laid on the institutional treatment of tuberculous children as a factor in the prevention of adult tuberculosis. Not only is the care of children with active pulmonary tuberculosis desired but, in addition, provision is sought for cases of bone, lymphatic and other forms of the disease. Preventoria for children with latent infection are also generally recommended as among the most efficient means for preventing adult consumption. All of us believe thoroughly in the value of these institutions and hundreds of them are already in operation. Some have now been established for a number of years, yet comparatively few data are available concerning the actual results of treatment and the experiences in adult life of the persons who have lived in them as patients during a portion of their childhood.

One of the purposes of such institutions is the removal of infected children from further contact with tuberculous parents. The amount of contact with such a parent necessary to produce infection has not been definitely settled. According to Bernard and Debré (1) none of 123 infants under their observation failed to contract the infection after six months of contact. Some degree of infection may, therefore, be expected in all children living for a considerable period with parents having open pulmonary tuberculosis.

The Grancher Institute (2), founded in France about eighteen years ago, takes healthy children, usually at least three years of age, from tuberculous families and places them in the country, in the families of healthy country people, where they pass their school life up to or beyond the age of thirteen. More than 2000 children have passed through the hands of this Institute. Only seven cases of tuberculosis have developed among them and of these four recovered.

[1] Read before the Medical Staff of the Lymanhurst School, Minneapolis, Minnesota, August 8, 1922.

In 1910 Goodall (3) reported the outcome in the case of 120 children with pulmonary tuberculosis discharged from Stony Wold Sanatorium during the first six years of its operation since its opening in 1903. Sixty-eight were between the ages of 6 and 12 and 43 between 13 and 16. Of those between 6 and 12, over 80 per cent were discharged "apparently cured" or with "disease arrested." Sixty-five per cent of the group between 13 and 16 were discharged with the same results. So far as Goodall was able to trace these cases, all were as well or better at the time of his report than they were at discharge. Making a liberal allowance for relapses, among those not traced, he feels sure that the results were as good as or better than among his adult patients.

Dunn and Cohen (4) report on 369 cases of tuberculosis in the Infants' Hospital in Boston as follows: died in institution, 194; died after discharge, 82; alive and well, 43; and untraced, 50.

The Westfield State Sanatorium for Children, opened in 1910 in Massachusetts, has recently established a follow-up system (5). To date the total number of children reported on is 438, as follows: died after discharge, 22; in good condition, 382; in fair condition, 24; and in poor condition, 10.

Available reports from institutions are not yet very numerous or complete, but this is not surprising as follow-up departments and supplementary home supervision for insuring lasting results from sanatorium and preventorium treatment are comparatively late developments in tuberculosis control.

The following summary of tables is presented with the hope that it will encourage the discussion of methods and means for keeping in touch with children who have lived in such institutions.

Nopeming Sanatorium was opened in May, 1912. The first child was admitted in June of the same year. In this report only children under twelve years of age on admission are included. One hundred and sixty-three have been discharged; of these, 19 died in the institution. Reports subsequent to discharge have been received from 144. Ninety were heard from in 1922. The last reports from the others were received at earlier dates, 19 being heard from in 1921.

Summary of tables

Subsequent condition of patients living at discharge

	NUMBER OF CASES	PERCENTAGES			
		Dead	Well	Living	Untraced
1. General summary	144	7	71	12	10
2. Family history of tuberculosis	77	6	77	8	9
3. "Suspects" in tuberculous families	20	0	80	5	15
4. "Suspects" in families without tuberculosis	16	12.5	56	12.5	19
5. Glandular tuberculosis	64	6	80	8	6
6. Pulmonary tuberculosis	30	10	60	20	10
7. Bone tuberculosis	18	16.5	56	16.5	11
8. Other forms of tuberculosis	3		100		
9. All tuberculosis cases (omitting duplicates)	107	7.5	72	13	7.5
10. Discharged 7 years or more	26	8	69	19	4
11. Under 1 year of age on admission	6	16.6	66.6		16.6
12. Under 5 years of age on admission	34	9	68	6	17
13. Five to 12 years of age on admission	110	6	73	15	6
14. In sanatorium less than 3 months	36	11	56	14	19
15. In sanatorium 3 months to one year	46	6	74	11	9
16. In sanatorium more than one year	62	5	79	13	3

COMMENTS

Table 1: General Summary: In interpreting the tables it is apparent that the numbers involved are too small to permit the drawing of many valuable deductions. If, however, similar reports from many institutions were combined, inferences of considerable value might be possible.

The cause of death in those dying subsequent to discharge is not necessarily tuberculosis. In this series, one child was drowned subsequent to discharge, another died from scarlet fever and another from diphtheria. In the case of certain others the cause of death was not ascertained. Beside those listed as "well" it may be assumed that some others included under the headings "living" and "untraced" are also in good health. It will be noted that about 83 per cent of those discharged living, were living or well at last report.

Table 2: Family History of Tuberculosis: In each case a member of the family had open tuberculosis. In most cases this was either the father or the mother and the contact was in all probability sufficiently long in each instance to have caused infection. (Von Pirquet tests were not made, as at present, in all cases. In 18 of these cases, however, positive Von Pirquets were secured). Over 85 per cent of these infected

children were well at last report. The report of the Grancher work in France and the excellent results obtained at the Ramsey County Preventorium of which Dr. Taylor can tell you also indicate that infected children from families in which there is open tuberculosis are not doomed. Many of them will remain in good health under proper conditions.

Table 3: "Suspects" in Tuberculous Families: Includes cases from tuberculous families in which the diagnosis of tuberculosis was not considered justified. All of these presumably had been infected, that is, had been exposed to tuberculosis, although negative Pirquets were obtained in 11 of these series. Four of the children died of other causes than tuberculosis in the institution; one died after discharge. The others are either well, living or untraced. Apparently the fact that these children remained in a tuberculosis sanatorium an average of 312 days or about 10 months has not yet resulted in their becoming actively tuberculous on account of infection or reinfection. Fourteen of them spent practically all of the time they were at Nopeming Sanatorium in the baby department where they do not come in contact with open pulmonary cases or with adult patients. Six spent all of the time they were here and two more a part of the time in the children's cottage. The children in this cottage come to the main dining-room to meals and entertainments. They come more or less in contact with adult ambulant patients.

Table 4: "Suspects" in Families Not Tuberculous: In none of these cases was a positive diagnosis of tuberculosis made. They came from families in which there were no known cases of the disease. Positive Pirquet reactions were obtained in 3 of them and negative reactions in 10. The majority of them were admitted as suspects, but subsequently showed no definite evidence of tuberculous disease and apparently had not been infected previous to admission. Four of them were under five years of age and spent most of their stay in the baby department; 11 were in the children's cottage and were brought more or less in contact with adult ambulant patients. The average length of stay of these children at Nopeming was 270 days or about 9 months. The follow-up shows one dead since discharge, the others either living, well, or untraced. None is known to have developed tuberculosis.

Table 5: Glandular Tuberculosis: Of the 68 cases in this series 43 were from families in which there was open tuberculosis. Four died in the institution. Sixty-four were discharged living. It is possible that fewer diagnoses of glandular tuberculosis would be made in this

series to-day, as it is becoming more generally recognized that a certain proportion of the chronic lymph node swellings, especially in the neck, are not tuberculous.

Table 6: Pulmonary Tuberculosis: Of the 36 cases admitted in this series 30 were discharged "*living*" including 12 with disease at least "*apparently arrested.*" Ten of these were well at last report and 2 are untraced. Sixteen were discharged with disease "*quiescent*" or "*improved.*" Seven of these were well at last report, 6 were living and 3 were untraced. Three of the "*living*" cases are known not to be doing well. In those in whom an "*apparent arrest*" was secured, all are now well, so far as traced. In only one case discharged as "quiescent" has there been a relapse.

Table 7: Bone Tuberculosis: The treatment in these cases has varied somewhat. Future reports under this and other headings should indicate whether hygienic treatment alone was used or whether it was combined with tuberculin treatment, orthopedic treatment, heliotherapy, etc.

Table 8: Other Forms of Tuberculosis: Of 6 cases having other forms of tuberculosis 3 died in the institution and 3 were well at last report.

Table 9: All Tuberculosis Cases: It will be noted that about 85 per cent of all the cases discharged with a diagnosis of tuberculosis were living or well at last report. This table does not represent the total of those of tables 6, 7 and 8, as duplicates are omitted.

Table 10: Discharged Seven Years or More: Although 23 of these 26 cases were living and well according to reports received subsequent to their discharge, in only 12 cases were reports received after the full period of seven years had elapsed. All of these 12 children were living and 6 of them were reported as well. In the case of the untraced and those from whom no recent reports have been received, we have no reason to suppose more recent reports would have been less favorable.

Table 11: Under One Year of Age on Admission: The ages of these children on admission were as follows:

Five weeks	1	Eight months	3
Two months	1	Nine months	2
Three months	1	Eleven months	1
Five months	1		

Four died in the institution, six were living at discharge. Eight were from families in which there were open cases of tuberculosis. Three of them had bone tuberculosis. Of these one died in the institution

and two were well at last report (1922). One case of pulmonary tuberculosis died in the institution. Two cases diagnosed as suspects are untraced. On discharge after 16 and 76 days, respectively, they were "*improved.*" Another diagnosed as "*suspect*" and discharged after 26 days' residence as *not* "*tuberculous*" died a year and a half later from drowning. Another "*suspect*" from a tuberculous family was discharged "*well*" after 661 days' residence and was well at last report (1922). His twin brother admitted as a "*suspect*" at the same time as he, when both were eight months old, died in the institution after 153 days' residence, in all probability from tuberculosis. Still another suspect two months of age on admission died after 6 days' residence.

Tables 12 and 13: The greater percentage of fatalities both in the institution and after discharge among the children under five years of age is at once apparent. Eighty-eight per cent of the children over 5 years of age on admission and discharged alive were living or well at last report as compared with 74 per cent in the case of those admitted under that age.

Tables 14, 15 and 16: The results obtained were apparently better in proportion to the length of the patients' stay in the Sanatorium. Only 70 per cent of those remaining less than three months and discharged living were alive or well at last report as compared with 85 per cent of those remaining from three months to a year and 92 per cent of those remaining a year or more.

CONCLUSIONS

While the number of cases involved in this report is too small to permit of broad generalizations, certain inferences may be drawn:

1. There is need for the standardization of forms to be used in securing data regarding the subsequent history of children discharged from institutions concerned in the treatment and prevention of tuberculosis. The results obtained in the various institutions should be comparable, and the records so kept as to furnish evidence on disputed points. Suggestions regarding such standardization should be correlated by the National Tuberculosis Association or some other national or sectional organization concerned with public health.

2. The need of more efficient follow-up systems and machinery for keeping in touch with discharged patients and reducing the proportion untraced is evident. This is desirable not only that better records may be secured, but much more for the reason that constant supervision and

encouragement of the children is needed to secure the best results from treatment. The good results obtained in the Grancher Institute work in France, where the children are under special care for a number of years between the ages of three and thirteen, is evidence of the value of prolonged supervision. Ex-patients from institutions for tuberculous children should probably keep in close touch with the sanatorium follow-up department or other field workers at least until adult life.

3. The value of prolonged residence at the sanatorium in securing permanent results may be inferred from tables 13, 14 and 15. In some way provision should be made for a longer stay in the case of each patient or for better supervision at home.

4. The preventorium makes it possible to provide care and supervision for large groups of infected children at less cost than sanatorium care. The providing of preventorium places permits the keeping of sanatorium beds for the patients needing special treatment. A certain proportion of the children concerned in this report would have been suited for admission to a preventorium.

5. If children are to remain in institutions concerned in tuberculosis control for long periods of time the importance of not neglecting to provide them every educational opportunity possible is apparent. Not to do so is to inflict a grievous wrong on the child. The example set by the City of Minneapolis in establishing the Lymanhurst School for Tuberculous Children is an excellent one that should be followed throughout the country.

6. The ultimate results in the treatment of pulmonary tuberculosis in children, in whom an arrest of the disease is secured in a sanatorium, appear to be nearly as good as in the case of adults.

7. Children from families in which there had been an open case of tuberculosis showed in this series a slightly larger percentage alive or well at last report than the entire group included in the study.

8. Children not showing definite evidence of active tuberculosis upon discharge from a Sanatorium apparently do not develop the disease later more frequently than other children.

The assistance of the Field Department of Nopeming Sanatorium, Dr. H. G. Lampson, Director, and Miss L. L. Christensen, R. N., Registrar, in the securing and compilation of the reports on which this summary is based is gratefully acknowledged.

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THE FUTURE OF TUBERCULOSIS WORK IN AMERICA

PRESIDENTIAL ADDRESS[1]

LAWRASON BROWN

To look intelligently into the future we must study carefully the past, but in regard to tuberculosis work history does not repeat itself, and the promise of the future lies not in a repetition of the deeds of the past but in a continuation, in a reinforcement of the fine spirit that has brought us thus far toward our goal, the eradication of tuberculosis as a widespread disease of all manner and condition of men.

The struggle against any disease can be approached from a number of different angles. For instance, it might be attacked from the point of view which would include first, state medicine, second, the efforts of private physicians and, third, a most important field, the coöperation of the laymen, trained and untrained. Again, we can divide all the workers in such a struggle into first, those whose aim is to help the immediate patient, to handle properly the immediate case, who endeavor to apply to each patient or case under their care the latest discoveries in the art of healing or of remedial care and, secondly, those whose aim is to discover new methods of diagnosis and treatment and of cure, discoveries which enable them to be of aid to untold thousands through the first group which applies them to the few hundreds of patients that fall under their care. The second group gains what rewards that fall to its share in the form of honors and academic positions, while the first group gathers where it can its often too scanty livelihood.

There must, of course, be some organization to bring together, to correlate, all this mass of endeavor and accomplishment and, furthermore, to distribute where it can best be used the knowledge gained from laboratory, from hospital and from field work. Those in charge of this central group must have critical and constructive faculties, must have an organization under their control which will enable them, with the least effort and as quickly as possible, to disseminate among all workers

[1] Delivered at the General Session of the Nineteenth Annual Meeting of the National Tuberculosis Association, Santa Barbara, California, June 20, 1923.

throughout our great country all knowledge useful in such a campaign and, furthermore, to inaugurate new measures. This is at present the task of our association, and how very well it has been performed is fully known to all of you.

Knowledge of tuberculosis has come slowly. For instance, it required twenty-three hundred years to prove that pulmonary tuberculosis was contagious, and even to-day, with all of our study, we cannot agree upon detail, for too much rests upon authority, too little upon ascertained facts. Much work remains to be done. For over eighteen hundred years we have known that sunlight, milk, good food and mountain air are good for patients with pulmonary tuberculosis. Here again we have added details and popularized ancient knowledge, but modern times have also made some real contributions. For over one hundred and fifty years we have been able to percuss a chest and for one hundred years we have had the stethoscope at our disposal, and yet, within the last fifteen years, many men have written upon the use of these methods (upon the production of râles by coughing) in such a way that one is led to believe that even many specialists in pulmonary tuberculosis were long unaware of the best technique.

I am recalling to you these well known facts simply to emphasize how slowly our knowledge of tuberculosis has accumulated. I could marshal other facts to prove how much work has been necessary to add a single minute, an almost negligible, new fact to the knowledge of tuberculosis. How many of you are aware that from 1893 to 1913 so many articles were written upon tuberculosis that the mere titles and references filled over 418 pages in fine print in the *Index Catalogue* of the Surgeon General's Library in Washington? To-day he who adds anything new to the knowledge of tuberculosis deserves the thanks of mankind.

So far I have referred to the clinical and laboratory work in tuberculosis. When we survey the public health and social work done in tuberculosis we realize that along those lines we have made wonderful strides. Such work has been a development almost entirely of the twentieth century. Its ramifications are so great, its work so well known to all of you, that it hardly seems necessary to discuss here in detail the most important addition to tuberculosis work made by this generation.

This then is briefly the position to-day of the struggle against tuberculosis. It might be amplified to cover many volumes but it will serve

us now as a basis for an attempt to read the future. I realize my limitations only too well to hope that any of you will do more than pause in your busy lives to consider briefly the few suggestions that I shall make.

Scientific laboratory work in tuberculosis has attracted relatively few men in America. The reason is not difficult to find. To-day all of us worship efficiency. With some of us it has become a fetish. We want results and we want them immediately. Alas, in tuberculosis work accumulates and results linger. Who to-day can conscientiously urge a brilliant young scientist to devote his life to the study of tuberculosis with its scanty rewards, in honors as well as in more tangible assets? With the same energy, the same skill, the same devotion in other lines of research he might claim recognition much more quickly and, as a result, reap more academic and other rewards than another equally brilliant student still pursuing that chimera of tuberculosis, the great discovery. For years I have been impressed with how little work in tuberculosis the great foundations were doing. Look through the lists of medical contributions and see how seldom a publication comes from one of our honored foundations. I am offering no criticism, for under the circumstances I very likely would do the same. But must the circumstances remain the same? I do not believe so. I am convinced that in a few years, if tuberculosis ceases to decrease as rapidly as it is doing to-day, we shall see established in America, preferably I should say (and regretfully I might add) in one of the large centres of population such as Chicago, Denver, Philadelphia, Cleveland, New York, Baltimore, San Francisco, Los Angeles, a foundation that will do for tuberculosis what the Rockefeller Institute and other foundations are doing for general medicine. Such a foundation needs a vast sum of money for many reasons. It must maintain a small but expensive hospital where the best and most modern treatment can be skilfully applied. It must have well-equipped and extensive laboratories. It must command the services of the foremost experts in many lines. They must be paid salaries commensurate with their position, and, furthermore, a retirement or pension fund must be provided for those who do good work, for it must be realized that they are truly devoting themselves to a search for the Holy Grail of tuberculosis. I do not imagine that such an institution can spring into being in one or two or three years. It must develop slowly, as men and problems arise, but behind it must be funds sufficiently large to enable it to reach its fullest development.

I am not unmindful of the great advantages that such a foundation would gain from a close affiliation with a university. Could this be brought about it would place tuberculosis work on a high plane, would enable the workers to keep abreast of many fields of endeavor, and to draw upon, to tap, so to speak, knowledge from many sources; and possibly a professorship in tuberculosis with its associate professorships and assistantships would drawn men who otherwise might not wish to devote their lives to such work. The advantages that would accrue to the medical student under such an arrangement are too patent for discussion.

In the meantime scientific work in tuberculosis has been wonderfully stimulated by the very meagre grants made by our Association through its Committee on Medical Research. Look at the work that has been accomplished. I know of nowhere in the history of tuberculosis where so much has come forth from so small an expenditure. If it is in the power of any member of this Association to increase the funds at the disposal of this committee he is casting bread upon the waters which will return a thousandfold. I can recall some years ago the slighting remarks about research work that passed current in some of our large cities. It was directed at medical work especially, and no doubt had some restraining effect upon contributions to literature that were known in common parlance as "potboilers." But thanks to several large foundations, there has gone forth over our land a very different outlook upon such matters. After nearly twenty-five years in tuberculosis and sanatorium work one thing at least stands out clearly, and that is that any group of workers, medical or social, who wish to do the best routine work for the patient or the case under consideration, must not limit their endeavors to routine work but, in season and out, must strive always for new and better methods. Routine is an excellent servant, but if she obtains the mastery all our life is bound in shallows and in misery. I have preached this, long and often, to my assistants and to my sanatorium colleagues. To-day I should like to emphasize its value for all workers, social or medical. I should like to see our Association recognize the value of social research by appointing a committee to stimulate and to guide it, and I should like to suggest that definite funds be set apart to enable those who have the spirit to pursue to a successful end such endeavors.

The complete eradication of tuberculosis, as I see it, must follow along one of two lines. First, by vaccination or by some other method we

must be able to prevent tuberculosis as completely as we can to-day prevent smallpox. If such a method fails us we must then discover some method of treatment that is as successful as arsphenamine and mercury are in early syphilis or quinine in malaria. The advent of either of these possibilities seems to-day as remote or even more remote than it did twenty-five years ago. Another possibility, of course, is that pulmonary tuberculosis is following the course of leprosy, ague, black death and typhus, and, like them, will slowly but surely recede until in countries with a high hygienic civilization it will be of slight importance. Time alone will reveal this to us, and we cannot abate our endeavors upon such a slight chance.

It is needless to call the attention of the members of this Association to the marked and increasingly rapid fall in the mortality rates in tuberculosis. They seem to have fallen in almost every community, and in some instances are certainly not entirely due to the antituberculosis campaign, for in these instances this campaign has been so little pushed that had the rates increased we would not have held the campaign a failure. What has brought about this marked decrease in the number of deaths? It is certainly not due entirely to the antituberculosis measures, as has been pointed out. It is surely due in part to them. "Amelioration of living conditions" is undoubtedly the cause of no small part of this decrease. The recent war seems to have had little direct effect, but the pandemic of influenza through which we passed a few years ago must be considered.

The future of the antituberculosis work in America, certainly as regards prevention, depends largely upon what this Association decides are the fundamental factors in bringing about the lessened death rate in tuberculosis. I should like to call briefly to your attention certain facts. The death rate from many diseases has been falling steadily for a number of years (smallpox, diphtheria, typhoid fever, malaria, children's diseases). But the tuberculosis death rate in the United States, Great Britain and Germany began to fall before the discovery of the tubercle bacillus and has been falling constantly, and, at an increasingly rapid rate, until the world war, and again since the war. In the United States in 1911 the rate was 159 per 100,000, and in 1921 only 99, a decrease of 38 per cent in ten years. But more recently some increase has been noted, for instance, in New York City. The cause of the decrease of the tuberculosis mortality in New York City has been carefully studied by one of our members, Haven Emerson

of New York. His thoughtful and suggestive article in our journal, the AMERICAN REVIEW OF TUBERCULOSIS, demands serious study from every worker in tuberculosis. He has analyzed so clearly the various factors entering into the decline of tuberculosis in New York, where dwell about 5 per cent of all the people of the United States, that it seems a waste of your time to restate the problem. Basing my remarks in part upon this study, I should like to state that in the future we must make renewed endeavors to improve and to standardize the diagnosis of all forms of tuberculosis, paying especial attention to the differential diagnosis, by which I mean the ability to state to a patient that he has not tuberculosis but some other affection. The importance of early diagnosis, of search for tubercle bacilli in the sputum, will always be of little avail until in the future they can be extended to the smaller communities throughout the land by the establishment of more tuberculosis clinics. In the future no less attention must be paid to the diagnosis of patients with slight pulmonary changes, but much more attention to those with extensive pulmonary changes and no tubercle bacilli in their sputum. In one sanatorium from 10 to 15 per cent of the patients admitted as early cases failed to have pulmonary tuberculosis, and in a hospital ward for advanced pulmonary tuberculosis 6 per cent had other diseases. The importance of the visiting nurse in helping to detect the nine active cases for each death can never be questioned. Here again the dispensary plays an important part.

We must increase our hospital and sanatorium facilities, and must decide whether we wish them to be used for diagnosis, for treatment or for education. Those who favor in the future shorter residence in sanatoriums, who hold that these institutions should be largely educational, call attention to one of the weakest spots in our campaign at present, but it is hopeful in that it is so widely recognized. The large number of sanatorium patients discharged with their disease arrested, who go home and relapse in some months, is appalling. In the future we must realize that the sanatorium has done most of its duty in arresting the disease, and that, from discharge on, the community, the board of health, the visiting nurse and the social worker have all responsibilities for keeping the "arrested" patient well. Why should not a patient with a scarred lung again fall ill when placed almost at once under the conditions which broke down his health at first? I said the sanatorium had done *most* of its work for the discharged patient. By that I meant that if by chance that patient begins to run down, con-

tracts a severe, persistent cold, the sanatorium should again accept him for a few weeks, and so prevent a relapse which not only may entail loss of chance for full recovery for the patient but also transform him from a careful, considerate patient in good condition to an advanced case, so weakened that he can take little if any precautions, and so becomes a menace to all his family and requires segregation. Is it not better for the future to set aside a certain number of beds for these patients, not only in sanatoriums but also in hospitals in large centres of population, and to readmit them as often as is necessary to keep them in good condition? I am not unmindful of the work already done along this line, such as fitting the arrested case for a return to work, in the Altro Shop by Mr. Stein and Mr. Hochhauser, in Philadelphia by Dr. Landis, in the Reco Shop by Dr. Miller and his associates in New York. The Potts Memorial Foundation at Hudson, New York, has decided to make a special study of this whole problem, working in close touch with the many sanatoriums and hospitals. The future importance of such work cannot be overemphasized.

In the future we must continue to enforce certain rules of community decency, such as those directed against promiscuous spitting, coughing without covering the mouth with a handkerchief, the common use of eating utensils, cups, towels, etc. These laws are most valuable, even apart from their educational value. Notification of tuberculosis as a communicable disease is helpful in many ways and must be pushed, even though but a fraction of the open cases are reported. The laws about food, especially those concerning the tuberculous contamination of food by flies, or even by patients, the eating of those parts of tuberculous cattle that are not diseased, are of far less importance than the law requiring the pasteurization of all milk that comes from noninspected, non-tuberculin-tested cows. This has apparently decreased the death rate from nonpulmonary forms of tuberculosis in New York in seven years by about 50 per cent, until to-day it is only one-third of what it is elsewhere, and has enabled Dr. Park to find only 16 per cent of the cases of tuberculous adenitis to be due to the bovine bacillus, whereas before 64 per cent was due to them. Pasteurization of milk and its products must in the future be pushed in all large cities where proper inspection and testing of the milk supply cannot be carried out.

So far we have spoken only of those prophylactic measures that prevent implantation of the tubercle bacillus in new hosts. It is only fair to say that some believe that slight infection, repeated at more

or less prolonged intervals, acts like a vaccination and prevents tuberculous infection from developing into tuberculous disease. Armstrong's work on the Irish and Italians in Framingham is very suggestive but needs confirmation from a larger number of observations. The marked increase of urbanization since the era of great industrial centres has apparently not increased tuberculosis, and some would have us believe that the increased opportunities for infection may have played a part in the declining death rate. The Jewish race has long been urbanized, and suffers less than others from tuberculosis. The recent large influx of Jews into New York City has no doubt played some part in its decreasing tuberculosis death rate. On the other hand, the recent migration of farm laborers and negroes into the cities would undoubtedly tend to raise, for a time at least, such death rates. Such facts as these emphasize the need for special study of the various elements of our population that suffer from a high tuberculosis death rate, and the development of new methods of attack. I am strongly of the opinion, however, that no evidence is yet at hand that would justify us in relaxing our efforts against infection from any source in order to further such a production of immunity. It is also very doubtful whether we have suceeded in reducing in any striking degree the number of individuals who have tubercle bacilli in their bodies. It is not difficult to see why this should be so, for each careless patient must infect a large number of other individuals. A single implantation in the body of the tubercle bacillus is sufficient to produce a definite tuberculin skin reaction, but I have long held that repeated infections, suitably timed and spaced, are necessary for the production of clinical tuberculosis. By the methods I have mentioned, so well known to all of you, we do, I am sure, reduce for many persons the number of infections below the limit necessary to produce clinical disease. Primary infection in America is most dangerous in babyhood, and superinfection during the age of greatest strain, from the fifteenth to the twenty-fifth years. The opportunities for infection and superinfection can be seen, when in New York City it is found that only 26 per cent of the patients were in institutions and 74 per cent of the known cases remained at home.

Now, if the tuberculous infections may be dormant for years, until such time as the bodily resistance is sufficiently reduced to enable it to pass from innocuous infection into active disease, we must, in the future, strain every effort to keep up a condition of good health which would thus tend to bring about increased resistance. In the future the struggle for

good health should begin before the baby is born, with the prenatal work. By breast feeding, baby clinics, nutritional clinics, school lunches, open air classes for anaemic, undernourished children, or even for most of the children, the good work must be kept up. I think the day will come when not only manufacturing plants, but all large schools, will have hygienists, practical as well as theoretical, whose duty it will be not only to teach but also to make continuous rounds of the plants or schools, to see that the workers or children have proper protection from hazards of all kinds, such as poor air, too low or too high room temperature, etc., and, further, are not taxed beyond their strength, which leads in children to faulty posture and its train of ills, and in the adult worker to lessened production.

Whatever critics may finally conclude about the Health Crusade work among children, it will always stand out as the best piece of pioneer constructive health work for children. In my opinion it should be steadily pushed and carried on from year to year. In time it may be advisable for us to combine our efforts with those of other organizations which seek the same aims. Whatever may happen in the future the National Tuberculosis Association must always be given credit for inaugurating an organized health movement for children, due largely to Mr. DeForest's untiring efforts.

The model tenements of London and New York are of value only when they are properly used, and proper use comes only with education, and education is assimilated only when those to be educated can put into practice the knowledge so gained, whether it be knowledge of cleanly personal habits, or cleanly communal habits, or of a better and higher standard of living. A higher standard of living implies less overcrowding, better houses, higher wages, better and more plentiful food, shorter hours of work, more time for play, more hours out of doors, and fewer and shorter periods of no work. Such conditions abolish worry, bring about contentment, lead to a lessened consumption of alcohol, though it is still difficult to say just how important a factor prohibition has been. We must not forget, however, the value of industrial and school health services, including the dispensaries.

All this leads us of course toward general public health work. Better water supplies, for instance, lead to less typhoid and dysentery, and so indirectly to less tuberculosis. I have no time to-day to go further into this subject, but I believe that every measure that protects the weak, strengthens the strong or cautions the careless, will aid in the prevention

of tuberculosis. Our future work lies so close to general public health work that we must coöperate with the public health officials in every way possible. We must also see to it that they get their share of credit in the winning fight against tuberculosis.

For years to come, no matter how successful our campaign may be, we shall still have those who, through carelessness, ambition, self sacrifice, debilitating conditions, will develop clinical tuberculosis that will demand treatment. In many instances only those without many social ties can leave home and work, and hence many sanatoriums are filled largely with younger patients (Drolet). The others remain at home or go for a time to day or night sanatoriums for training, and then resume work if possible. This is a problem that needs much study in the future. The Home Hospital, established by some members of our Association, has pointed out one, and an excellent, solution of the problem.

The future of this treatment (of those afflicted with pulmonary tuberculosis) is of considerable importance to all those who have to do with the state or municipal treatment of this disease, for if a change in treatment is likely to occur in a few years, we must take it into consideration when we build our extensive and expensive structures created for this purpose. As I look back over the past twenty-five years I can see a great development in the housing of patients for treatment, but the actual treatment of the pulmonary group of these patients, by far the largest number, has changed little except in detail, and in the later stages when complications supervene. In the future more attention will be paid, I feel sure, to a certain degree of creature comforts. The patients will be allowed to remain more in well-ventilated rooms, for it will be accepted that a change of temperature, of surroundings, is as important in the daily routine as it is now considered in the ordinary life of the individual. I mean by this that seven or eight hours out of doors in colder weather will produce the tonic effect we desire upon the nervous, cutaneous and muscular systems, and through them indirectly upon the lungs. We will some day feel that the amount of fresh air that produces the best results varies within certain limits, and depends directly upon the individual concerned. To-day we treat all patients with the fresh air bath, made as nearly continuous as possible, in the same way we used the Brand method of baths for typhoid fever when I studied medicine twenty-five years ago.

On the other hand, the treatment of the so called surgical or extrapulmonary tuberculosis has undergone rapid changes in the last two decades. Thanks to Rollier chiefly, and to Pryor, Hyde and LoGrasso among others in this country, those sufferers are to-day subjected far less frequently to disabling surgical operative treatment than formerly. I am not unmindful of the value of extensive surgical knowledge in the treatment of this group by heliotherapy, but the development in the future will, I believe, extend along the more conservative lines, and less surgery will be done. It behooves those who now have in charge the erection or maintenance of state and municipal sanatoriums for pulmonary tuberculosis to study carefully this problem of nonpulmonary tuberculosis, and to begin at once to make provision for it in all sanatoriums for pulmonary tuberculosis. That it will be a near future step can be foretold by anyone who has visited Perrysburg. It is also possible that in the treatment of these patients there will be added to heliotherapy radiotherapy, deep X-ray therapy, which some claim even to-day produces just as good, and even quicker, results than the usual heliotherapeutic measures.

The treatment of pulmonary tuberculosis as applied to early cases has changed little for some years except in the stricter use of methods of rest. I predict that in the future we may become a little stricter still in the treatment of early cases; in the hope that we may prevent the too large percentage which now advances to the later stages, and in many instances to ultimate death, from so doing. In the sanatoriums, those which do not now do so will soon begin to review at the end of two months of treatment, and at intervals thereafter, all patients, early or late, who fail to respond at once to the ordinary hygienic-dietetic treatment. This will soon be seen to be especially important for those with small or no funds, and for those with dependents where years cannot be given to the "rest" cure.

The treatment of the more advanced cases of pulmonary tuberculosis received a wonderful stimulus when artificial pneumothorax was introduced some years ago. In the future we shall consider it, I believe, earlier, and seldom allow a case early or late to go down hill, to show definite and marked advance of the disease, determined by physical signs and by X-rays, without resorting to this form of treatment, though it may be only palliative. The work of some of our members has improved this method of treatment. Recent work has enabled us to treat, often most successfully, that most trying of all complications of

artificial pneumothorax, pyopneumothorax. This will abolish many of the past fears concerning this method. Thoracoplasty is an outgrowth of collapse therapy.

Twenty years ago the most dreaded, and almost always fatal, complications of pulmonary tuberculosis were meningeal, laryngeal, and intestinal tuberculosis, and diabetes. The prognosis in meningitis is still as hopeless as intestinal tuberculosis and diabetes in patients with pulmonary tuberculosis were considered some years ago. To-day the problem of the treatment of diabetes has been largely solved. We are able to diagnose early, and often to cure, secondary intestinal tuberculosis. Laryngeal tuberculosis has been conquered by voice rest, direct and indirect heliotherapy, natural and artificial, and by electrocauterization. Who to-day dares to say that in the next few years meningitis will not also yield to skilful treatment, applied early and properly?

The search for a cure for tuberculosis still goes on, but we are apparently no nearer to it than we were twenty-five years ago. Specific treatment by the various tuberculins is still extolled, but many have failed to accomplish with tuberculin what they were led to believe they might. I still feel that in tuberculin we have possibilities for the cure of early cases when we shall be able, by newer methods of physicochemistry or by other procedures, to select certain cases suitable for treatment, and to gauge and to space aright the doses. Chemotherapy may yet do for tuberculosis what it has done for syphilis, and give us a means of curing early cases, but so far there is no light ahead. Those who are older in tuberculosis work can count on their fingers and toes the "cures" for tuberculosis they can recall. Within the last few weeks two or three new "cures" for tuberculosis have been published in the newspapers. Our executive office is constantly replying to inquiries upon such matters. I feel sure that Doctor Williams would appreciate any information about the workers who publish such claims, and about their claims, which any of our members possess. Furthermore, it seems to me that all such claims should be investigated by our Association through a special committee appointed for this purpose. By no means should this work be turned over to our Committee on Medical Research, for they have enough to do.

I have left until the last the discussion of the future of our Association. Every member knows that thanks to it the antituberculosis campaign in America is further ahead than in any other country. It has bound

together workers in all fields of tuberculosis. Had I the time I would not have the strength to recount its accomplishments nor you the patience to listen to me. Along what lines lies its future work?

Of education, of propaganda, there is as yet for the Association no end in sight. Most of the ground has been sown, but in places the seed has fallen upon stony soil, in others weeds have sprung up and choked it. It needs to be watered, carefully tended, encouraged, and in the end the harvest will be beyond our expectations. The physician of to-day may need little further education in tuberculosis, but the physician of the future, the medical student of to-day, requires thorough education in the diagnosis and treatment of all forms of tuberculosis. This problem has received careful consideration by our Association which needs your constant help in its solution.

During the life of our Association the greatest contribution, as I have stated, to tuberculosis work has been the awakening to the fact that tuberculosis was not wholly a medical problem. The sociological side has been wonderfully developed. More remains to be done. Willing hands are ready. We must supply the means for further attacks. But I can see the day not so far distant when the sociological workers will be in the position of many physicians engaged in tuberculosis work some years ago. The older methods at that time seemed to have been worked out, and not until new ideas were introduced did the work again forge ahead. New methods of attack must be sought, for which we shall have to depend upon renewed and further scientific and research work which only those with prolonged training can give us. The National Tuberculosis Association must ever depend for guidance upon its scientific workers and advisers.

We must take serious thought about our relations with other national health organizations. How much should we coöperate with them when our work overlaps? As I read the future we must work more and more with every agency that shows a proper spirit of fellowship and cooperation. We must begin to realize that we too must yield in part to further best the great work in which we are all so eagerly engaged. Petty jealousies will arise, but those who foster them will be swept aside by the rising tide of a great work moving toward completion. We must all further realize that to-day the antituberculosis campaign is a public health matter of wide importance. Every measure for the improvement of the general health helps reduce the tuberculosis toll and suggests the question: Should we look forward to a time when the work of the National

Tuberculosis Association can safely be turned over to the national, state and local health authorities? Those who think we should ought to read the recent report by Winslow and Baker in the AMERICAN REVIEW OF TUBERCULOSIS. As I see it, such a step can never be taken until we place the position of health officer on such a plane that the community will not only honor and trust him, but will gladly and willingly pay him a salary that will recompense him and enable him to devote his whole time to preserving the health of the persons entrusted to his care. How many health officers have had special training? How many can afford to study when we pay them such a pittance? In the meantime, what should we do to bring about concerted effort? I feel that we should get behind and push every attempt toward such an end, and, furthermore, that we can safely leave it for the present in the hands of our Managing Director and Executive Committee to suggest the best course.

Criticism was brought against the workers in tuberculosis about ten years ago, that if only the measures they had at hand could be applied to any community the death rate from tuberculosis would be very materially affected. You are all aware of the generous offer made to the Association some five years ago by the Metropolitan Life Insurance Company, through its Vice-President, Doctor Lee Frankel. It has resulted in the Framingham Demonstration under the guidance of Doctor D. B. Armstrong, with which we are all familiar. The demonstration was to include, as the work progressed and broadened, an endeavor to find out how much tuberculosis existed in the community, how best to detect it and to treat it, to develop along with these measures a reasonably adequate general health program, to ascertain its cost, and to see if the community could be led to assume it.

What has it accomplished? What does it point to for the future? It has shown that probably one-half of the active cases are undetected, and not under control. It has increased the notification of cases, and of the discovery of new cases (largely through an efficient consultation service conducted by Doctor P. C. Bartlett). It has led to the treatment of more patients in hospitals and sanatoriums. It has led probably to a decrease in the mortality from tuberculosis that is genuinely significant. It has increased the expenditure for health from 40 cents to $2.30 per capita, and finally it has brought about the greater Milbank Demonstration which is endeavoring to apply to a county in New York State (Cattaraugus), to a city (Syracuse), and to a part of New York City the data obtained in this demonstration.

Figures can undoubtedly be brought to show that certain other communities have, without any special antituberculosis campaign, enjoyed just as great, just as rapid a fall in death rate, but, on the other hand, the "control" towns about Framingham have had only a slight reduction in their tuberculosis mortality. Here again, though the evidence at hand is not conclusive, every worker in tuberculosis welcomes with open arms the great experiment in the purchase of health brought about by the Milbank Foundation. Such work must be done in order to demonstrate its value to the country at large, and your Association, and some of its members, have had much to do with it.

Finally, I should like to stress one very important thing that, as time goes on, as the pressure increases for local expenditures, as many phases of our work cry out for funds, we are all of us always in danger of overlooking the national scope of our work, that is, of not heeding the cry to come over into Macedonia to help. Few can to-day picture to themselves the annoyance, the interference with free intercourse which existed when New Jersey, for instance, taxed the products of New York, and New York taxed those of New Jersey. But with free interstate commerce and intercourse have come other burdens, particularly in regard to health matters. Sparsely settled states have small funds and relatively increased expenses, but the tuberculosis patients can wander into these states from others where funds are sufficient to conduct an efficient campaign, and so bring to them new foci of disease. We are to-day indeed our brothers' keepers. In health matters we can know, if we strive aright for our high aim, no west, no east, no north, no south. Those who are weak we must support until they can stand alone. Too many of us have only recently learned to walk. I hope the day will never come when in New York or in California, in Washington or in Florida, we shall lose that sense of national helpfulness that has always existed in tuberculosis work, and restrict our altruism to the confines of the municipalities and states in which we live.

A CLINICAL STUDY OF THE GASSED EX-SOLDIER WITH SPECIAL REFERENCE TO PULMONARY TUBERCULOSIS

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Since 1915, when the first poisonous gas of the war was used against the French troops in Flanders, a great deal of interest has been devoted by the medical profession to the whole subject of gassing. Isolated cases of gas poisoning, occurring in industrial plants and chemical laboratories, have been known for many years, but, owing to their infrequency, they created little comment and no particular interest among general medical practitioners. Since the war, however, and particularly since our own soldiers have suffered from gassed lungs, our attention has been more closely drawn to the subject. To-day, seven years after the first gas patients appeared in Europe, we must still admit that we do not know the answer to many pertinent questions which have arisen concerning the diagnosis, treatment and prognosis of these patients and particularly those questions in relation to pulmonary tuberculosis. A great deal of excellent laboratory and pathological material on this subject has appeared, but little, in this country at least, of a clinical nature which might help us, when called upon to make differential diagnoses between gassed lungs and conditions which simulate them very closely.

It is my object to discuss the question from the clinical rather than the pathological standpoint. If, in presenting my experience with these cases, covering a period of nearly four years, I can contribute anything of clinical interest or value concerning them, I shall consider the purpose of this paper fulfilled.

I make no attempt to review in detail the history of gas warfare and all of the interesting points concerning it, but, for purposes of greater clarity, briefly summarize essential facts concerning the nature of poisonous gases, their mode of action and their pathological effects. In preparing this summary, I draw freely upon the literature already published and desire to acknowledge a special indebtedness to a paper presented

before the Chicago Medical Society, in January, 1922, by Colonel Gilchrist, of the Army Medical Service. The main purpose of this paper, however, is to point out the results of personal experience, however inconclusive they may be, in an effort to differentiate satisfactorily between those cases which may be clinically classed as the results of gas alone and those which may properly be classed as due to gas plus tuberculosis.

My personal experience began in a base hospital in France, located a considerable distance behind the lines, where patients were received after all doubtful cases had been eliminated and sent back to duty. Since my return from service, I have seen these cases continuously, for diagnosis and treatment, in my connection with the Veterans' Bureau and in the care of a number of gassed men suffering from pulmonary tuberculosis at the Palmer Tuberculosis Sanatoria and in private practice. This last group constitutes a special one in which closer study and more detailed observation have been possible than in any of the other groups.

The use of gas for offensive and defensive warfare goes back many hundreds of years before the Christian era. History records that pitch and sulphur were used by the Athenians against the Spartans about 430 B. C. The smudge pots used by Caesar's legions, to drive the defenders from the besieged walls, were the same in principle as our modern suffocating gases. However, the mustard gas of to-day is to those smudge pots of antiquity what the modern Zeppelin is to the old fashioned smoke balloon, the result of a great many years of experiment, trial and error. We have no better instance of scientific barbarism brought down from bygone ages to modern times than the gases used in the past war. Barbarous as this method of warfare undoubtedly is, unless there is more security in the present World Agreements at Paris and Washington than in previous similar agreements, it is scarcely to be expected that so potent an offensive and defensive weapon will be overlooked in the future. Are we not justified in attaching considerable importance to these gases and our clinical results, for this reason if for no other? To regard these cases as isolated ones, a byproduct of this past war, of temporary interest because we will not see them for long or meet the condition again, would seem a grave mistake. In this past war, preparedness of the medical profession has proved itself of equal importance to any other branch of the service. According to Colonel Gilchrist, about 27 per cent, or some 70,500 casualties of this past war,

were due to gas, and some 1,221 cases terminated fatally. If we consider the persons who have died since the armistice, in civil life, from tuberculosis, bronchiectasis, pneumonia and other pulmonary affections, caused or aggravated by war gas, which are not included in the above figures, it seems reasonable that the fatalities would be much higher.

Without attempting any exhaustive study of the chemical and physical nature of gases, it may still be of some interest to review briefly the nature of some of these poisons in order that a better understanding of the clinical findings may be gained.

War gases are of several groups, and may be classified according to their action on the body. The following is based on notes taken from the first gas school held in Paris for American medical officers, and gives, I believe, a fairly clear picture of the various types. However, it is well to bear in mind, not only that the classification of gases overlap, but that, at times, more than one gas has been received by the same individual. All gases are irritant to some extent. All irritant gases may be suffocating in sufficient concentration. Most gases have some toxic properties. They are listed, therefore, with the above in mind and classed according to their most striking characteristics.

Group 1: Asphyxiating or Suffocating Gases: This group includes a number of gases which produce death by suffocation of the patient, either because the gas is irrespirable or because of an alveolar exudate preventing absorption of oxygen by the lungs. The most important and best known of the group were chlorine and phosgene. The former was experienced more by our allies than by the Americans. Its expensive manufacture and difficult application, combined with other disadvantages, caused its replacement by phosgene before the Americans arrived in full force upon the scene. The physical nature of chlorine made its uses as a gas cloud particularly easy. It was transported in large pressure tanks to the field, and, when the wind was right, the gas was released by means of valves; and, being heavier than air, it was carried along the ground over the intervening space between the trenches by the wind. There are several disadvantages to this method: first, its cost of production; second, impossibility of using it against high ground as a cloud; third, its dependence upon weather conditions; and, fourth, the attendant danger to the attackers in case the wind should shift.

Phosgene, a lighter gas, needs chlorine to add persistence, but it lends itself more readily for use as a shell gas, and hence possesses many

of chlorine's good points and does not possess its objectionable features. Warning of the approach of cloud gas could be given much quicker and better than the approach of shell gas. Wind and weather played smaller parts in its use, and not only could it be thrown further from the attacking force as a shell but its diffusion was more certainly assured should it be blown back by the wind.

These two gases and the type they represent were of particular value to an attacking force which intended shortly to occupy the position gassed. Their action was swift and deadly and, owing to their instability, the attackers were comparatively safe in crossing their objective an hour or so later. It was for this reason that these two were given preference for preparing an advance, though their use was by no means always so confined. At times they were preceded by lachrymators or sternutators, which would induce the defenders to remove masks, thus paving the way for the more deadly sequel.

The mode of action of this asphyxiating group was in general about as follows: Immediately following the gassing, or in the first stage, there was reflex stoppage of respiration and cardiac inhibition, probably of vagus origin. The result was immediate collapse, with fall in blood pressure, cyanosis and general weakness. If the concentration was sufficient, death ensued. Most of this action was reflex, from the upper and lower respiratory tract, as evidenced by the fact that when this stage had passed there was a second stage of comparative normality during which few signs or symptoms could be found. It was not unusual for men to be sent back to duty during this period with disastrous results. The second stage was variable, both as regards its time of appearance and duration. If the patient had been severely gassed some polypnea with relatively slow pulse was usual. The sufferer might be blue or gray, the latter cases being much more serious and regarded by Colonel Gilchrist as more likely to prove fatal. Twenty-four to thirty-six hours after the initial gassing, the third stage set in, characterized by marked dyspnoea, prostration and cyanosis, or shock with falling blood pressure and signs of general collapse. Cough was paroxysmal and painful, with profuse expectoration of bloody foam and mucus. Death usually followed within five days, the patient literally drowning in his own secretions. Death which took place later than five days was usually due to pneumonia. Patients who recovered often showed gangrene of the extremities and various haemorrhagic and thrombotic tendencies throughout the body. Pathologically speaking, the lesion was more

severe in the ultimate anatomic divisions of the lungs, such as the air cells and alveoli, the bronchitic disturbance characteristic of mustard gas being either absent or relatively unimportant.

In those dead of phosgene or chlorine there was an agglutination of red blood cells in the lungs, combined with oedema and necrosis of respiratory epithelium, superficial or deep, depending upon the severity of the case, with exudate into the alveoli. In later cases, massive infiltration with giant cells and leucocytes and with thrombosis of the vessels led to a picture similar to true inflammatory consolidation. A great many patients died from secondary bronchopneumonia, but whether toxic or infectious in origin was difficult to decide in any given case.

Physical signs in these cases, in the latter part of the second stage or first part of the third, showed varying grades of cyanosis and dyspnoea with low temperature and slow pulse. Prostration was marked and the facies anxious and drawn. As the condition advanced, cough with burning and discomfort on inspiration made its appearance. The pulse became more rapid and weak, and in many cases there was a culmination with shock and cardiac failure. The cyanosis was replaced by pallor, giving the so called "gray case" previously mentioned. Rapid emaciation was common.

Chest findings in this stage were often surprisingly meagre. Inspection usually showed all the accessory muscles of respiration being brought into play in an attempt to increase lung space. At times the percussion note was impaired from about the second to the fifth rib anteriorly, and the fourth to the eighth spine posteriorly. More often a tympanitic quality to the percussion note was noticeable, usually posteriorly. Definite dulness was uncommon and when present was usually due to bronchopneumonia in the area affected. Breath sounds were usually found diminished or absent, especially if edema was established. Fine and medium râles of all types could be detected throughout the chest. Heart dulness was definitely diminished in extent. It is well to note here that the râles were neither so large or numerous as is common in mustard gas cases. The picture here was that of an alveolar rather than a bronchial process. Severe cases often showed signs of patchy bronchopneumonia, particularly behind, below the angle of the scapula which, pathologically, were found to be definite patches of true bronchopneumonia or haemorrhagic infarcts interspersed with areas of atelectasis.

Dyspnoea and cyanosis responded, if at all, to oxygen, rest and cardiac or respiratory stimulation. Once having given patients of this type

access to oxygen tanks, it was found next to impossible to discontinue it, so great was the need. Oxygen, except in high concentration, fails to reach the blood by reason of the thin layer of fluid overlying the alveoli, but there is apparently little resistance to the passage of carbon dioxide from the blood. The condition is an oxygen starvation. In the case of mustard gas, not only is the absorption of oxygen affected, but also the excretion of carbon dioxide. Dilatation of the skin vessels was not nearly so marked in the chlorine or phosgene as in the mustard gas cases, so that typical cases of either are not easily mistaken. However, clinical differentiation of the two conditions, due either to mixture of the gases or to other factors, was not always possible.

In these cases treatment was directed to supplying oxygen and combating cyanosis and bolstering up the heart and circulation until the acute stage had passed. Oxygen inhalation and venesection and cardiac and respiratory stimulants were used in selected cases. Supplying water to these patients was often difficult and, though it might be expected that large quantities might favor edema of the lungs, it was nevertheless urgently demanded for the support of the circulation. Steam or benzoin inhalations proved disappointing and failed to give the expected relief, though they were tried. The feeding problem was difficult at times owing to irritation of the tract and to the gravity of the cases. Shock, which was exceedingly common, was combated according to the approved methods. All of our methods, while appearing to give some measure of relief, were often hopelessly inadequate, and it seemed to be the general impression that the ultimate outcome was settled by the initial gassing on the field before we ever saw these cases.

The after-effects of this type of gassing seem to have been less than those following mustard gas, probably because of the fact that the necrosis of tissue was not so extensive or deep as was common after mustard gas. Clinical experience with these cases, since leaving the army, has been limited to a few, and I am by no means certain that these were true chlorine or phosgene cases without admixture with mustard gas. I have yet to find a case of tubular bronchiectasis which I am certain was due to either of the suffocating gases. By far the greater number of gassed men that I have seen since the army experience has been classed as "mustard gas" or "mixed," and the after-effects of mustard gas obscure the picture. Phosgene cases have shown practically negative findings, and chlorine cases merely slight harshness of breath sounds fairly generally distributed.

Group 2: Toxic Gases: This group I shall class as "toxic" and mention it only in passing, as I have had no experience with it except with carbon monoxide which leaves no permanent signs. The group includes hydrocyanic acid, arsenic trichloride, stannic chloride, arsine, the cyanogens of chlorine, bromine and, lastly, carbon monoxide.

The last named gas was often the result of the dugout charcoal brazier, which smothers the patient at night, and was not unusual in enclosed machine gun pits after prolonged use. A few hours in fresh air was usually sufficient to clear such patients as were taken from the scene alive, and ten days usually saw them no worse for their experience. Many lives were needlessly sacrificed by carelessness on the part of the men with these charcoal braziers.

Group 3: Irritant Gases: This group includes lachrymators, sternutators and the vesicating gases of which mustard gas is the most important. The latter gas at one time created such havoc among the armies of our allied nations that it was thought that it might end the war in favor of the Central Powers. The lachrymators and sternutators were not fatal except in high concentration, but they were exceedingly serviceable in forcing the removal of masks or in the disabling and retarding of laborers and other workers in the service of supply. Among the more important members of this group are to be found bromacetone, halogen derivatives of benzine, ethyliodoacetate, and phenylcarbylamine. The action of this group was mainly the production of intense irritation of the mucous membranes of the eyes and nose, leading to swelling, edema and exudation. This group has recently been advised for use in civil life, by troops engaged in riot duty.

Corneal ulcers and loss of taste and smell were frequently seen following these gases, and, though not usually attended by serious consequences, the resulting disability was often severe. It should be remembered that mustard gas has a very marked effect in the lachrymatory field, but differs from the other gases of this class in its more important pulmonary action and for this reason mustard gas is considered separately. The more severe cases of irritant gas poisoning, particularly those in which the lungs were involved, led to pictures so similar to those due to mustard gas that clinical differentiation was impossible.

Mustard gas, a true vesicant, is by all odds the most important member of the gas family. Chemically, it is dichlorethylsulphide, a brown liquid which volatilizes slowly and hence is a most persistent gas. Its

discovery is credited to Victor Meyer, in 1886, and also to him belongs the credit of the first experimental evidence of its respiratory-irritant characteristics.

Mustard gas, a liquid at ordinary temperature, must be volatilized before becoming of any importance as a respiratory irritant. The liquid itself produces severe burns when applied to any portion of the skin or mucous membranes, leading to an appearance similar to the mustard burns which occur from ordinary mustard plasters and packs. Its action is rather slow, but difficult to stop when once started, and the end result may be anything from superficial redness to an eschar. The droplet burn was known among medical officers as "splash burn" and was often deep and extensive.

The physical nature of mustard gas is such that it lends itself particularly well to shell use. Blown into the air from its glass container within the projectile, a large part of the liquid is immediately volatilized, the remainder falling in droplets into the earth and continuing to saturate the air with gas for days and weeks after the original shot. Water contaminated with mustard irritates and burns the flesh wherever it touches, and many an isolated soldier has paid a dear price for his drink from a contaminated shell hole. Instances are not lacking of whole platoons and larger units suffering from water contamination by the drug.

It may be easily seen from the foregoing that the casualties of mustard gas were not only in the front line troops, but extended clear back even beyond the zone of fire, falling heavily upon the service of supply and kindred organizations. A retreating army, scattering mustard gas in its wake, may obtain casualties days and weeks after its evacuation, and mustard gas therefore takes first rank in the retaliating measures of a retreating force.

To the peculiar quality of mustard gas, of adhering to garments and equipment generally, are due many casualties among the workers who handle not only gas but general battle casualties, since mustard gas may be carried in this way back to the hospitals. One of our own base hospital staff operating a fluoroscope, where the cases were brought some miles by stretcher to him, was severely gassed in this manner. He was operating in a warm room where gassed and wounded men were brought for the detection of foreign bodies, and, owing to the pressure of circumstances, many soldiers still wore the same clothing in which they were picked up from the field. The gas which arose from

the clothing of these men, extending over many hours, was sufficient in quantity to produce in the operator eye, throat and bronchial symptoms of a severe nature, and to keep him from duty for nearly three weeks. There were many instances of wounded men with mustard gas-saturated clothes being severely burned, on the way back from the front and near-front, during the time before Americans became conversant with the methods of handling these cases.

An interesting incident of this kind, occurring in my own wards, illustrates the point. During the St. Mihiel drive an infantry sergeant was wounded in the thigh by a machine gun bullet and taken directly back to the base hospital by railroad, after a first aid dressing in one of the field hospitals. He arrived the following day and was placed in a surgical ward. Owing to the number of patients received the same night, he was not bathed, though his clothes were removed, and he was put to bed to await his turn. The following morning he complained of general itching of the skin, and examination showed a brick red color of his entire body, particularly marked in the axillae and groins. Scarlatina was suspected, but the subsequent history and character of the skin proved beyond doubt that mustard gas was responsible. The history showed that he had lain about two hours in a shell-hole before having been picked up, and it is probable that this was the source. He showed no other evidence of gas except slight reddening of the conjunctivae and lachrymation.

Complete removal of mustard gas-infested clothes with clipping of the hair was at times not sufficient to prevent severe scalp burns, some of which proved exceedingly stubborn. I distinctly remember one patient who lost the superficial skin of his entire scalp down to the hair line, and that in spite of the fact that we noticed the presence of the gas on the appearance of the first erythema. Washing with soap, alkalies and various solvents failed to arrest the steady march of the process and his suffering was intense. It is probable that his head was wet at the time he went through the gas, and the concentration was probably very high. His body, eyes and respiratory tract were scarcely affected. It is my belief that the gas must have been carried with the moisture beneath the skin surface, along the hair and into the scalp glands, and hence could not be reached. Scrotal and axillary burns were the usual accompaniment of body burns, and were often severe and persistent and particularly disabling on account of their sensitiveness.

The history of these surface burns is interesting. They may be of any grade, from mild erythema to eschar, depending upon the concentration of the mustard gas and the length of time it is allowed to act. There is first itching and redness, resembling the rash of scarlatina, followed by exudation of clear watery serum which cakes on the surface when allowed to dry. Removal of this cake at this stage takes the superficial epithelium with it. After the condition advances, vesication takes place and, by coalescence, large thin-walled bullae may be formed. Death of tissue in any quantity was distinctly uncommon and usually limited to small areas where droplets had fallen and had not been removed; so called "splash burns." Severe deep tissue burns were also seen in cases where bits of shell, rock, splinters or other material, saturated with mustard, were blown deeply into the flesh and hence could not be reached in time to prevent serious damage. Wounds so produced were extremely painful and difficult to handle. Pigmentation of the skin appeared usually about the time healing first became apparent and in some cases was extremely marked. Many of the darker-skinned Caucasians showed extensive pigmentation as the only signs of having come in contact with this gas. The widest individual variations imaginable were evident. The blonde and fair-skined individual tended more to blister, and pigmentation was not marked or it appeared later. The darker-skinned individual, of the Mediterranean type, pigmented very heavily. The negroes usually showed nothing except possibly erythema until vesication appeared, and, on healing, absence of pigmentation was noticed. This pigmentation is apparently of the same nature as that seen occasionally as the result of mustard plasters, and well known to those who use this method for counterirritation. Most of it clears entirely within twelve months, but some cases among my records have shown it after two years. Loss of superficial epithelium apparently caused no disability, but I have noticed a very definite tendency in the older and more severely burned individuals to pyogenic skin infection, such as carbuncles and boils. Severe cases, with deep seated lesions, also showed some loss of hair, but I have been surprised at seeing this sequel so infrequently.

The treatment for these skin burns is practically the same as for any other similar superficial burn. Exclusion of air, bland ointments, and prevention of infection were the methods used. Dakin's solution was also used where the lesions were not too extensive; and 10 per cent boric acid ointment proved of inestimable value in most cases.

The action of mustard gas on the conjunctivae is essentially the same as that seen on any other external part of the body, modified by local conditions. The irritation and lachrymentation led to intense photophobia, lasting sometimes for weeks; headaches were persistent and severe; local conditions favored swelling of the tissue involved which was usually marked. Superficial ulceration occurred occasionally. All efforts of the physicians were centered on the protection of the cornea and relief of pain, the latter often extremely difficult. Alkaline washes, boric acid packs and mydriatics were the usual methods employed, combined with reduction of the light.

It is with some hesitation that I approach the subject of the action of mustard gas on the respiratory tract. First, there are many points yet to be determined concerning its action and too little is known concerning the ultimate outcome of the cases. Records of a sort were necessarily kept in the hospital on our acute cases, but they were valueless so far as detailed study was concerned.

From our whole group of more than a thousand severely gassed cases, some very definite general impressions present themselves without the clarity which characterizes the composite picture. Perhaps the composite picture may ultimately give us a clearer view than the personal impressions of individual cases can afford. I cannot give statistics or figures, unfortunately, to prove my impressions, but trust that the reader will take my statements in the light of a general résumé of cases seen, and regard my remarks in the light they are intended.

As I look back now on this group of severely gassed men, seen three and four years ago, my first and strongest impression is not strictly a medical one. It is really that of the extremely pitiful condition presented by them when they first appeared for care. Most of them were blistered from head to foot and scarcely able to speak above a hoarse whisper, and some of them not at all. Their eyes were beefy red and swollen, with uncontrollable tears streaming down their faces. The light cut and burned whenever they were placed in its full glare. They were racked by paroxysms of coughing, followed by futile attempts to remove the blood-streaked tenacious froth from their lips with bandaged hands. Many were scarcely able to eat because the irritated throat produced reflex vomiting when disturbed by food. Some of them were unable to stand, sit or lie in comfort, or to read or smoke.

Most of our severely gassed patients reached us by stretcher from the fields through the evacuation hospitals, by way of boxcars or hospital trains. The majority had remained long enough in the previous hospitals for a diagnosis to be established and for the worst of the shock to be combated, and they were received by us for that long period of after-care and convalescence, which was necessary in the mustard gas cases. It is easily seen, therefore, that our cases belonged, in the main, to the more seriously sick group of mustard gas casualties from which the milder ones had been weeded out. On an average, the patients reached us from five to seven days after they first reported for treatment or were picked up, as the case might be. We therefore received these patients about the time bronchopneumonia became evident or would be expected to appear, and our experience with them amply supported this assumption.

The physical examination as regards the respiratory tract revealed a fairly characteristic picture at this time. At times it was possible to pick out from the surgical wards unrecognized cases among patients who had been sent to us as wounded. While, at first glance, the latter fact appears relatively unimportant, it was at times of very definite value to us, particularly as regards the administration of general anesthetics for operative work.

The temperature, if taken with care, was almost universally found elevated at entrance, though even in cases of definite gas bronchopneumonia it was usually not high, the range usually being from 99° to 101°F. The absence of high temperature in these cases was considered by us as of fair prognostic import, and prognosis was considered much more grave when a temperature of 102° or more was discovered.

In contrast to this, the pulse rate was usually found quite slow, counts of below 70 being the rule and 50 to 60 extremely common. Even when definite bronchopneumonia, with dulness and raised leucocyte count, was found, the pulse was uniformly about ten beats lower than would ordinarily be expected in other respiratory diseases with the same grade of fever. This low pulse rate often persisted throughout the stay of these patients in the hospital and has been noted many months afterward in the examinations in civil life. This condition tends to clear up gradually, as subsequent examination of these patients has shown. Rapid pulse, found earlier in these patients, was considered distinctly unfavorable. An interesting fact about this bradycardia is its reaction to mild forms of exercise and excitement. While at rest, marked brady-

cardia was the rule, and yet it was found on exercise that the pulse was markedly irritable and unstable. This circumstance again has been noted in the older cases since discharge, with gradual return to normal after a period of months or years. Whether any of these neurocirculatory phenomena after gassing may or may not be permanent, I am unprepared to say.

The respiration was found accelerated in practically all cases, whether bronchopeumonia was present or not, and we were thus deprived of one of our valuable differential diagnostic findings. It was often difficult in these cases to decide just when bronchopneumonia first set in. It is evident that more diagnostic stress had to be placed on the physical signs of consolidation, higher fever, leucocyte count, X-ray pictures and the character of the sputum.

Inspection usually revealed a picture closely resembling an asthmatic attack. Varying grades of cyanosis were often found, extending, in the more severe cases, to the mucous membranes, finger tips, ears and cheeks. Aside from any direct mustard gas change the superficial skin vessels were generally dilated over the entire body surface, imparting to the body a dusky or brick-red color. Vasomotor instability and anxiety were frequently noted.

The chest was found extended to its greatest capacity, with accessory muscles of respiration brought into full play, and yet the patient was quite short of breath at rest. Breathing was shallow and rapid, with paroxysms of coughing. The apex beat of the heart was seldom visible or palpable, being covered by lung border.

Percussion, except in cases of bronchopneumonic consolidation, usually revealed slightly impaired resonance, or at times tympany, from the third rib to the base in front and from the fourth spine to the base posteriorly. Definite dulness was distinctly uncommon, except in cases with definite bronchopneumonic consolidation.

The breath sounds in these areas were usually diminished, with numerous râles of all types, particularly behind. Patients without definite pneumonia at entrance almost universally developed some signs of patchy consolidation at some time during their stay with us. X-ray of these cases showed the usual picture of scattered bronchopneumonia except that, in general, both lungs suffered about equally and the consolidation apparently was deeper seated than is usual in nongassed cases. Massive consolidation was of rare occurrence.

Small pleural exudates were not uncommon and were usually of the serous type. I do not remember seeing empyema as the result of gas pneumonia more than three or four times. In two of our cases haemolytic streptococci were found in the exudate.

The most marked symptoms were the cough, prostration and dyspnoea, the former being particularly resistant to all our therapeutic measures with the possible exception of benzoin inhalations. At least some measure of comfort was gained thereby. For the dyspnoea of these cases oxygen was not attended by any marked results, probably because the condition was more bronchial than alveolar. Feeding was often extremely difficult because of the irritated condition of the throat and larynx. Severe coughing fits, producing reflex vomiting, were responsible for most of our trouble and we were puzzled by it until we hit upon the plan of breathing exercises before meals. A fit of paroxysmal coughing was thereby precipitated and the patient usually cleared enough mucus from his bronchi to give a temporary respite of an hour or so.

The usual course of these cases was about six weeks in bed with a low, irregular fever, leaving in its wake a chronic cough with considerable purulent sputum. The latter persisted far into convalescence and was one of the most difficult symptoms to relieve. Gas cases in private life, one and two years after their army experience, still show this symptom, and, judging from their course, under various therapeutic measures, combined with their X-ray and clinical course, I believe that a mild grade of bronchiectasis is really the pathological lesion which underlies the condition. More will be said on this subject under another heading. Since these patients do not die I have had no chance to verify my findings by autopsy.

Loss of weight was progressive and marked in all our severely gassed cases, and weight was only slowly regained, even after definite convalescence was established. In some cases emaciation was extreme, particularly those in which gastrointestinal symptoms were marked. This was due, I believe, to a number of different factors, not the least of which was the difficulty in keeping these patients supplied with sufficient quantities of water.

During the febrile stage, sputum production was very large, often reaching as much as a litre a day. During the first week it was usually serous with some frothy or blood-stained mucus intermixed. As the disease advanced and infection set in, it became more and more purulent.

The blood disappeared, except for rare streaks, and by the time convalescence was established the sputum had the appearance so often found in chronic, low grade, purulent bronchitis. During the stage of bronchopneumonia, there was more of a tendency toward the tenacious type, but definite prune-juice sputum was distinctly uncommon in our experience.

The patients were usually allowed up about ten days after the last fever, but graduated exercise was not begun until a week or so later. The majority were evacuated about this time to make room for others, so that their course for the next six months must be mostly a matter of history, as we were not permitted to see these patients except in a few individual cases.

One group of these patients deserves special mention because of the high mortality observed among them. I refer to a number of gassed patients who were accidentally exposed to influenza en route to the base hospital. The mortality in this group was terrific, reaching nearly 50 per cent in the twenty cases studied. The pneumonias of this group were the most severe and extensive, the fever the highest, and the clinical course more fulminant than any that we saw. It is reasonable, I think, in these cases to compare them to the severe bronchopneumonia sometimes observed after a severe case of measles; in other words, a virulent infection on a previously damaged membrane of low resistance.

The bacteriological study of the sputum of these cases was disappointing. Several strains of streptococcus and staphylococcus and other common mouth bacteria were found, but not with sufficient frequency to form any definite conclusions. Influenza bacilli were found in less than half the pneumonias from the gas-influenza group mentioned above. Haemolytic streptococcus was found a number of times in our fatal cases, as well as in a few which recovered. It is possible that they added something to our mortality list.

The whole group of gassed men was made up of difficult material from both the medical and surgical standpoints. The great majority were also badly burned externally, and many were severely wounded in addition. If those who have not seen such cases can imagine a three to six-week bronchopneumonia complicated by resistant, extensive skin and mucous membrane burns, often with extensive wounds, into which foreign bodies and mustard gas had been blown by shell explosions, some of our difficulty may be appreciated. The wounds in these cases

were particularly distressing and difficult to handle, since in only a few cases could all severely damaged tissue be removed by debridement.

Pathologically, the victim of mustard gas apparently suffered the most severe damage in the upper respiratory tract and bronchi. The lesions becoming less severe as the alveoli were approached, the trachea, bronchi and bronchioles therefore bore the brunt. The situation here is slightly different from that of a chlorine-phosgene group in which the alveolar picture predominates.

With the foregoing picture of the gassed man in mind, I did not find it easy to reconcile myself to the comparatively meagre signs found in a similar group of patients examined in civil life, a year or more after the armistice. I sincerely regret the fact that in my study of these men, there had to be a gap between their condition in army hospitals and in civil life. This lapse is partly filled by their clinical histories, but these histories are by no means complete. There is also another factor which enters into the consideration of this latter group of cases, which was not present in the former. The latter class does not represent, unfortunately, the ordinary run of post-war gassed cases, since those who have been sent to me for examination and care by the Public Health Service or the Veterans' Bureau have usually been referred because of tuberculosis or because some other severe pulmonary sequel has been suspected or diagnosed. They have therefore been sent for differential diagnosis, and the rank and file, except those who have applied to the Government for pensions, have not been seen. Whatever figures I may have, therefore, are obviously likely to be one-sided and might lead one astray unless this fact be recognized. For this reason, this latter group of gassed patients in civil life will be studied more particularly from the standpoint of differential diagnosis and more especially as concerns pulmonary tuberculosis.

When first called upon to make this differential diagnosis, I believed that it would not be particularly difficult. I expected the history and physical and laboratory findings, combined with X-ray, to settle the question for me in practically all cases without more than the usual allowance for error. After two and one-half years' work in this field I know that such is not the case. I have found that the most exhaustive study, with hospital observation, temperature records, repeated physical and sputum examinations, together with the most painstaking search and study by X-ray, have often left me in doubt. The element of time

has cleared a few doubtful cases, particularly those which turned out later to be definitely tuberculous, but there still remains an uncomfortably large group which is carried on my records as suspicious, and which is being watched assiduously for breakdown, unequivocal signs or positive sputum, which will settle the matter beyond all possible doubt. This latter group, in general, is being treated as tuberculous, in spite of the questionable diagnosis, on the sound theory that when in doubt it is better to err on the side of safety.

Of all the differential diagnoses to be made in the thoracic field, I know of no other more baffling or difficult of accomplishment than this. Among other members of the profession, both general practitioners and specialists in thoracic disease, I have found, on inquiry, a good deal of same opinion. The records of gassed cases of the Palmer Tuberculosis Sanatoria show an almost 50 per cent error in diagnosis, and what little I may add to the sum-total of differential diagnostic points may perhaps help some other man confronted with a similar problem.

Case histories being the basis of all differential diagnosis, I desire to describe three cases as fairly representative of the problem, working from the purely gassed case without complications, through the suspicious cases, to the positive gas and tuberculosis cases, discussing differential points with these cases as a clinical background.

Case 126: W. K., age 25, coal miner, white, married. *Family History:* Father, mother, six brothers and five sisters living and well. No deaths or chronic disease in immediate family. *Personal History (prewar):* Always enjoyed excellent health with no serious disease except pneumonia with good recovery. Usual childhood diseases without complications. *Army Medical History:* Entered October, 1917. Carried on his service in the artillery (with good health), until severely gassed on Armistice Day. Was sent by stretcher and train to Base Hospital 27, where he remained two months for treatment of burns and lung condition. States that he had considerable fever during this time and was told he had pneumonia. Returned from the hospital to his command and remained with it on light duty until discharged at Camp Grant, April, 1919. States that he was coughing at the time of discharge and was not well, but failed to mention this fact to his examiner because he wished immediate discharge.

Post-War Medical History: Did not attempt to work for a month and, on his return to mining, in June, found he could scarcely remain at work a full day on account of marked shortness of breath on exertion. The dust of his occupation brought on frequent paroxysms of coughing which made him dread

his work and kept his "lungs sore," as he expressed it, "clear to the bottom." Coupled with his shortness of breath was a lack of staying capacity, so marked that he could load less than half his daily prewar amount of coal. Severe headaches, becoming worse as the day progressed, made their appearance. In spite of his disability, he remained at work the following winter, during which he had several attacks resembling influenza, with severe prostration lasting a few days at a time and during which the sputum production, usually noted only in the morning and evening, became profuse. During his short attacks of illness, cough was severe and persistent. There were sharp pains in both the sides and front of the chest. The sputum, usually yellowish and thick, was occasionally streaked with bright red blood, though there had never been definite haemoptysis. Various medicines were tried for the cough without avail.

He was examined in my office February 28, 1921, his chief complaint at this time being dyspnoea on exertion, cough, headaches and weakness. Temperature 98°, pulse 72, respiration 20. Weight 134, average 135. Florid type with anxious facies; marked vasomotor instability; sweating of palms and axillae; no definite cyanosis. Slight, patchy, brown pigmentation of trunk and upper arms at site of old skin burns. Breathing apparently difficult at rest. General examination exclusive of chest, negative. Chest wide and deep; inspiratory position. Expansion 2 inches. Percussion note hyperresonant over both lungs. About 2 cm. excursion on both sides. Kronig isthmus four finger breadths on both sides. Superficial heart dulness diminished. Auscultation showed high-pitched, slightly prolonged expiratory sound throughout central portion of both lungs, not so marked at apices or bases, except right apex. Scattered throughout both lungs were numerous medium râles. X-ray showed slight general increase in peribronchial tissue, particularly toward the hilum, and lower central portions of both lungs, more marked in lower than upper lobes. Sputum showed no tubercle bacilli, but many streptococci and pus cells. Blood pressure and urine normal. *Diagnosis:* Chronic bronchitis, the result of gassing.

Change of occupation was advised, to get the patient away from the dust of mining, and reëxamination at three-month intervals. He was placed in vocational training and reported as instructed. During the past year, he has been treated along the general lines of a nonspecific pulmonary infection of low grade, with postural drainage twice daily and one or two short courses of respiratory tract vaccine, and such medicines as seemed necessary at times for the control of cough. He has steadily gained weight and strength. All symptoms except occasional cough have disappeared and he has been in better health and spent more days at work this past winter than at any time since his original injury. The sputum has disappeared entirely.

This patient furnishes a typical example of chronic, low grade, infectious bronchitis engrafted upon a war gas injury of the lungs. I do not believe that he has at any time had an amount of destruction in the bronchi which would warrant a diagnosis of tubular bronchiectasis, which has been met with in some of these cases. I doubt whether tuberculosis has ever been, or is now, a factor in his disability. The repeated febrile attacks, becoming less frequent as the time since the initial gassing lengthens, are worthy of note. Such attacks as I have seen have been similar to influenza of mild grade, and one or two have been severe enough to warrant a diagnosis of low grade bronchopneumonia, though definite consolidation has never been found. As seen in this case, the history of gassed men in convalescence is really that of chronic, nonspecific lung infection. Their treatment should be directed along the lines usually employed in this condition.

Time is a distinct factor, in my opinion, in the improvement of these cases and the majority, with the possible exception of those who develop bronchiectasis, would tend to clear up with no other therapeutic measures. It is questionable in my mind whether these cases will ever return to completely normal health. They are handicapped for certain occupations and it is probable that their acquired tendency to acute pulmonary infections may be a distinct factor in shortening their normal expectance of life.

Case 534: R. K, coal miner, white, married. *Family History:* Father killed in mine accident; mother and one sister living and well; no chronic family disease so far as known. *Personal History (prewar):* Always enjoyed good health. No serious illness prior to service. *Army Medical History:* Entered service May 6, 1917. Remained in good health under training, until severely gassed, June, 1918. Sent by stretcher to hospital and remained three months, following which he returned to his company and was discharged in April, 1919, without disability. Was given light duty in his organization after his return to duty, because of considerable cough and lack of staying power.

Post-War Medical History: Found he could not keep pace with his fellow-workers and was forced repeatedly, by cough, fever and general malaise, to lay off for considerable periods of time throughout the year. In the fall of 1920, indigestion after meals appeared, his cough became so severe as to cause frequent vomiting after meals and he began to lose weight rapidly. Stopped work during the summer of 1921, with a ten-pound gain in weight and general improvement in his condition. Returned to work again in September, with a gradual reappearance of all symptoms.

He reported for examination in October. His chief complaints at time of examination were general weakness, shortness of breath on exertion, cough, evening headaches and indigestion after meals, palpitation of heart and loss of weight. Temperature 99°, pulse 96, respiration 22. Weight 115 pounds, usual 138. Appeared ill and coughed frequently during examination. Slight tremor and signs of vasomotor disturbances during examination. Sallow with slight yellow tint to the skin. General examination negative except as regards chest. Thorax long and narrow with slight right shoulder droop and some degeneration of the right shoulder and neck muscles. Fremitus more marked than normal over the right upper chest. Percussion showed moderate dulness above fourth rib and fifth spine on right side. Left side normal. Auscultation showed harsh interrupted, bronchovesicular breathing above third space and spine and prolonged expiration above the fourth space and sixth spine, with numerous scattered crepitant and indeterminate râles over affected area, extending posteriorly to base. On left side there were a few indeterminate râles, fairly generally scattered throughout lung, most of which were relieved after cough. Suggestive blurring of Kronig isthmus on right side, but little narrowing. Heart normal. X-ray plates showed rather definite increase of peribronchial shadows in both upper lobes, more marked on right, of greater density above third rib than below. They were regarded by the radiologist as distinctly suspicious of a tuberculous process. Sputum negative for tubercle bacilli. Blood pressure, systolic 120.

While a diagnosis of pulmonary tuberculosis might be made on such evidence as this, it is hardly adequate in a previously gassed case. On the other hand, one would hesitate to discharge this case as nontuberculous. Observation in a hospital for a week showed afternoon temperature of 99.2° on two occasions, with persistent high pulse rate but no other information beyond two additional negative sputums and a negative Wassermann. Has this man pulmonary tuberculosis? All of the findings might be due to gas plus chronic bronchitis, and yet there are certain things which point definitely toward tuberculosis. The persistent loss in weight, high pulse rate and chronic indigestion make me feel that there is something more to this case than mere gassing. Degeneration of muscles on one side speaks distinctly for a more serious diagnosis. This man was therefore placed on a tuberculosis regimen at home, with regular rest hours, ample diet and open air sleeping. He will return for frequent reëxaminations. Lacking further evidence on the outcome of this case, I shall consider him and treat him as a case of chronic pulmonary tuberculosis, and withhold absolute diagnosis until positive sputum, pleurisy with effusion, pulmonary haemorrhage or some tuber-

culous complication settles the matter beyond all possibility of doubt. If he clears up, as I suspect he will, the diagnosis will remain "suspicious." In either case, the treatment outlined seems rational. I do not regard as ruled out the possibility of an upper lobe bronchiectasis, but consider it unlikely.

Case 9: R. V. S., age 23, white, single, photographer. Nativity, New Jersey. *Family History:* Father and one brother dead, cause unknown, otherwise negative. *Personal History:* Always well and carried on occupation without trouble. *Army Medical History:* Entered service October, 1917, and had no sickness until severely gassed and wounded at Chateau Thierry. Was not sent to a hospital, but remained with his command until the St. Mihiel drive when he was sent to Base Hospital no. 117, in September, 1918, remaining until November, 1918. During his stay with the company, he coughed severely and lost weight. In November, after the armistice, he was sent home in a casual company and was discharged February, 1919.

Post-War History: He rested for a month after discharge and then returned to work at former occupation, but weakness and general malaise became so marked that he gave it up and reported to the Government for an examination. He was advised, in November, 1920, to stop all work and rest, which he did.

He was examined in my office in June, 1921, at which time the following points were noted: His chief complaints were cough, weakness, loss of weight, dyspnoea, indigestion, nervousness and persistent blood-streaked sputum. Temperature was 98.8° in the morning, pulse 88, respiration 20. Weight 130, average 145. Apparently ill, with dry, sallow complexion. Coughed frequently during examination, and showed marked signs of vasomotor instability. The finger nails were long and curved; the hair dry and atrophic. General examination, exclusive of chest, negative. Chest long, flat type, with definite degeneration and droop of right shoulder girdle, and extensive lag on inspiration on right side. Percussion showed definite dulness to third space and fourth spine on right side, with definite narrowing of Kronig isthmus and blurring of inner border. Left side normal. Auscultation showed harsh interrupted, bronchovesicular breathing with numerous crepitant râles throughout right upper lobe, increased on cough, more marked posteriorly. The left side showed slight prolongation of expiration opposite third spine, with few crepitant râles. Sputum negative on two examinations and positive for tubercle bacilli on third, taken a week later. X-ray showed infiltration of both hilum regions and middle portion of right upper lobe, without definite tubercle formation. A diagnosis of chronic pulmonary tuberculosis was made and sanatorium care advised, mainly for educational reasons. This, however, was refused. The patient was therefore placed as nearly as possible on sanatorium regimen at home and was seen at weekly intervals. After six months

he was gradually got out of bed and exercise was begun. At the present time he is in a quiescent state, and though not well by any means, has made a substantial gain. Influenza last winter caused a loss of some ten pounds in weight, which he has not yet made up. He has been forced by financial needs to take up a part of his occupation again and is now working practically a full eight-hour day. Complete recovery, clinically, is not expected in this case because of several extrapulmonary factors, chief among which are his mental attitude toward the disease and the fact that he will not accept prolonged sanatorium training.

Taking the preceding three cases as fairly typical, we find the following points of interest: (1) Cough is common to all three, hence of little value in differential diagnosis. Shortness of breath and weakness are also of little value, except for the fact that weakness is more pronounced in the tuberculous. (2) Loss of weight of any magnitude is distinctly in favor of tuberculosis. (3) Headaches and blood-streaked sputum may be found in both the gassed and tuberculous. (4) Definite haemoptysis, which none of these three cases showed, should be considered tuberculous until proved otherwise. (5) Fever, especially if persistent, is evidence for tuberculosis, and positive diagnosis of tuberculosis in a gassed case is risky in its absence. (6) Pleurisy, though not present in any of the above cases, calls for a regimen of tuberculosis treatment, and pleurisy with effusion stands close to positive sputum in diagnostic significance. I have not found pleurisy in any simple, gassed case in the absence of pneumonia, and, while not common in the gassed case with tuberculosis, I still regard it as a highly suggestive diagnostic sign.

Of the physical signs differentiating the two conditions, the following seem most important: (1) Dulness of an apex or contraction and blurring of the Kronig isthmus, found a year or so after gassing, have not been seen by me in simple gassed cases, and their detection therefore, at times, give valuable aid. The pathological changes of gas are found usually toward the central and lower portions of the chest, rather than the apices; our old rule concerning apical lesions, therefore, holds good where gas is concerned. (2) Harsh breath sounds are often found in gassed cases of moderate severity, but bronchovesicular breathing, outside the regions where it is normally found, has not been seen by me in gassed cases except during the stage of acute pneumonia. (3) Laboratory tests of the sputum and blood reactions, while usually disappointing, may occasionally throw some light on the question. The presence of albu-

men in the sputum may be found in either the gassed or gassed and tuberculous. I hesitate to rely upon a positive complement fixation test and consider it distinctly inferior to the Wassermann test to which it is comparable. (4) Radiography has been of value in differential diagnosis, but even here serious mistakes are possible. All tuberculosis is not apical, and gas changes, hazy and uncertain as they usually are, may be grafted upon an old healed tuberculosis, creating a very confusing picture and giving rise to a false opinion as to the clinical significance of the extensive tissue changes present. It is one of the strongest links in the chain, however, and should never be neglected. Small cavities at times appear which physical signs have failed to show, constituting evidence of the utmost significance.

Some interesting information as to the differential diagnosis of gassed cases may be seen from the following figures, taken from the first 600 cases examined for the United States Veterans' Bureau during the years 1919–1920. The group does not represent the general run of gassed men, but only those suspected by the local examiner as being tuberculous, because of some suspicious history or peculiar findings.

TABLE 1

Total number of ex-soldiers examined	600
Total number of gassed men	196
Suffering from gas alone	89
Suffering from gas plus tuberculosis	107

TABLE 2

	GAS	GAS PLUS TUBERCULOSIS
Gassed men coming from tuberculous families	3	18
Haemorrhage in history	27	46
Febrile	2	68
Afebrile	87	39

In the tables, the following facts are worthy of note: (1) One-sixth of those diagnosed as tuberculosis have come from families of known tuberculous taint, while more than one-third of those remaining have a suspicion of tuberculosis in the family history, contrasting with the fact that of those diagnosed as gas alone one twenty-ninth showed tuberculosis in the family history and only one forty-third of those remaining had suspicious family histories.

Without going into the family tuberculosis problem, it seems evident to me that many of those persons who developed tuberculosis probably would have done so regardless of the gassing. They were very likely to have been heavily infected or actually tuberculous before their army service. It scarcely seems reasonable, therefore, to place the responsibility for their breakdown on war gas any more than on heavy drilling, overstrain, overwork and other factors incident to military service. Second, while recognizing that histories of haemorrhage in persons applying for government compensation cannot always be relied upon, it is still interesting to note that 44 per cent of the tuberculous claimants gave this history, as against 30 per cent of those diagnosed as gas without tuberculosis. It is also interesting to note that in no single case found to be suffering from gas alone has the history of large haemoptysis been given. In all cases where large haemorrhage has appeared in the history, tuberculosis has been diagnosed and subsequently proved to my satisfaction. Third, the presence of fever has come to be regarded as a most valuable sign in our differential diagnosis. A febrile state was found in 63.5 per cent of the tuberculosis cases, as against 2.2 per cent of those not found tuberculous.

SANATORIUM EXPERIENCE

A study of the records of the Palmer Tuberculosis Sanatoria, at Springfield, Illinois, up to December 31, 1921, has presented several distinct surprises. Excluding cases now in the Sanatoria and cases in which gassing does not appear in the discharge papers, we find there have been 27 gassed men sent for treatment. Of this number, 13 were found to be definitely tuberculous, 8 having positive sputum at entrance and all being febrile. One patient died five months after entrance, this being the only case that had progressed to the stage of cavity before admission. One has returned for a second course of treatment after an original stay of eight months. One other case has applied for readmission on account of reactivation of his disease. The remainder are in training centres of the Government or at home under the care of their local physicians.

Of those diagnosed as gassed only there have been 14, and their average stay for study and observation was ten days. None of these cases was febrile. None has since died and there has been no subsequent return for treatment. So far as I am able to state, all these patients have done well, and none has broken down with tuberculosis.

While recognizing that this group is far too small for definite conclusions, there are still some interesting inferences to be drawn from their study. Chief among these is that 14, or more than half, definitely diagnosed as tuberculous, failed to prove tuberculous on closer study. Only one conclusion can be drawn from such a record and that is that other physicians are experiencing something of the same difficulties in diagnosis that we have had. Another point of interest is the low mortality rate from tuberculosis among gassed patients. It is possible to explain this on the ground that ex-soldiers are getting earlier and better diagnosis than the average civilian, with the consequent earlier hospitalization, or that the gassed tuberculous soldier has some physical advantage over those not gassed. Whether this latter is true or not is open to question, but it is worth considering.

Again, reference to case histories shows that by far the greatest number of errors in diagnosis were made from one to two years ago. Recent cases show relatively few mistakes. It seems evident to me that the medical profession has gained considerable experience in this differential diagnosis within the past year, and that probably, when the patient is known to have been gassed, he is now receiving more thorough study before hospitalization. Another fact of importance is that gassed cases have cleared up gradually and, as time goes on, they show fewer and fewer physical signs.

Perhaps the most interesting observation is that this group of gassed tuberculous persons will average in better shape, both as to the stage of disease and systemic disability, than any similar group of nongassed cases, over the same period of time, that we have had at the Sanatoria. Considerably more than one-thirteenth of our government cases, exclusive of the gassed, have been in the advanced class. The course of these gassed cases has been, in general, of a low grade of activity and of more chronic indolent type than is usual in the other government patients. It has been our experience that their prognosis is better and that they hold their gains and less frequently return for further care than our other government patients. Add to the above facts that none of our gassed tuberculous cases was of the acute ulcerative type, nor has there been in any of them a single case of tuberculous pneumonia, meningitis, pleurisy with effusion, empyema or tuberculous kidney complication, and I believe that we must look further than simply earlier diagnosis for the reason. Our feeling here is that the tendency to sclerosis found in

gassed cases may in some way be responsible for the more favorable outlook. Long clinical experience, which none of us has had in this condition, combined with close pathological study, is necessary before this question can be definitely settled.

Absence of throat complications has been rather a surprise, in view of the severe mustard gas action on this part in the acute cases. No eye or skin changes have been noted except tendency to boils, as previously mentioned.

Experience with these gassed men without tuberculous complications in civil life has not been extensive, but there have been enough of them to show some of their general tendencies. Two years ago acute cough and respiratory infection were very common among them. As these cases have become older, these infections have become more and more rare. Since Springfield lies largely in a mining district, my attention has been drawn more particularly to the occupational side of the problem. As a class, these men have not done well in the mines and other dusty occupations.

Many of these gassed men have migrated westward, and I am sure more will follow, in hope that a change of climate may give them the comfort they seek. If, in ten years, we find a considerable percentage of these patients residing in comfort in the west or southwest, we may concede a point to the curative value of climatic change.

After some little study of the gassed ex-service men, I believe it is open to question whether gas *per se* is a real factor in the development of the post-war tuberculosis, and, while I am unprepared at this time to state definitely that gassed cases developing tuberculosis do better than others, I am inclined to that impression at this time.

In general, my inferences based upon four years of close observation of gassed and gassed-tuberculous patients are:

1. In the gassed patient there is a general and steady tendency toward recovery with decrease in physical disturbance. The exceptions are those cases in which the gassing has been very severe with considerable tissue damage. For these the ultimate prognosis is still in doubt.

2. War gassing has little to do with the development of later pulmonary tuberculosis.

3. The gassed patient who later develops tuberculosis usually runs a less severe course with less tendency to serious complications than the nongassed individual.

4. Differential diagnosis of the after-effects of gassing from pulmonary tuberculosis is fraught with difficulties, but it is becoming more satisfactory through the recognition of rather dependable diagnostic points, of which temperature, progressive loss of weight and tendency to haemorrhage are the most important.

5. The ultimate outlook for gassed patients, either with or without tuberculosis, is far from settled. The effect of gassing on life expectancy can be determined only by further experience. The adaptability of gassed patients to the various trades and vocations is not yet satisfactorily determined. The future physical disability of gassed persons and the amount of compensation or disability to which they will be properly entitled cannot yet be determined.

CHAULMOOGRA OIL ESTERS IN THE TREATMENT OF PULMONARY TUBERCULOSIS

(A Preliminary Report)

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It was a natural step, following the work on leprosy, to apply the ethyl esters of chaulmoogra oil to tuberculosis, since the biochemistry of these two pathogenic acid-fast bacilli is so similar. The method of application was the primary obstacle. Here experience was the same as that which followed the introduction of tuberculin. Before its physiological action was understood, the latter was used with great enthusiasm. It was not long, however, before a tremendous reaction in the opposite direction occurred and its use was discredited. Then came a period of quiescence, and finally another period of rehabilitation in which the dose was modified and its use found some substantial support among phthisiotherapeutists.

Chaulmoogra oil has gone through this evolutionary cycle, even though its use in tuberculosis is scarcely three years old. Having this experience clearly in mind in the beginning of my work with chaulmoogra oil, I determined not to be discouraged by the apparent failures of others or by untoward reactions, but to alter and fit the dose to the individual as determined by the clinical reaction. I further decided that, if the treatment was to be generally adopted, all such observations, to be of any value, must be made by physical examinations, using, of course, only such laboratory aid as is at the command of every one.

The adoption of a method which would meet the critical investigation of others was of the utmost importance. I therefore decided upon the following plan, in which the personal equation, from the clinical and laboratory standpoint, would remain as nearly as possibly constant, and the likelihood of error be reduced to a minimum.

All patients were given a thorough physical examination, which was controlled by the whispered voice sound. Any method, however, in which the examiner feels most proficient would be acceptable, as most men develop for themselves a definite technique of physical examination

and diagnosis which they follow in preference to a suggested routine with which they are not familiar. Beside the physical examination, the weight, general appearance, temperature, and the patient's own observation of his sensations were carefully recorded and analyzed. The sputum was examined in every case, and no patient whose sputum did not have acid-fast bacilli was treated. No discrimination or selection was made with regard to the stage of the disease. When the diagnosis was established, the plan of treatment was outlined as nearly as could be determined to fit the case. The method of arriving at the plan was not an arbitrary one, but was adopted from observation and experience. A routine handling of all cases is a point I would emphasize and warn against.

In conjunction with the chaulmoogra esters, all cases were placed upon individually selected diets, exercise and the other usual procedures adopted and recognized as being of benefit in the treatment of tuberculosis. In tuberculous adenitis, deep X-ray therapy was used, and surgery, in the use of the suction apparatus, when the lymph nodes had suppurated and pointed. All of the cases having a rise in temperature were given bed-rest. All hemorrhage cases were kept in bed for a period of from three to four weeks. If fever was present after that period, bed-rest was continued for as long as was necessary to establish with reasonable surety that neither the temperature nor the hemorrhage would recur. Those cases without contraindicating complications or symptoms were treated as ambulatory. The contraindications were high temperature, active hemorrhage and a pulse rate of 100 or over.

The first dose administered was 3 cc. of the ethyl esters by mouth. As it was evident that patients would not tolerate that form of administration, the intramuscular route was adopted. The objections to this method were intense pain and induration at the site of injection, which was given high in the gluteal region. The pain radiated down the leg, and the induration became reddened and showed an inflammatory reaction in which a localized tissue necrosis was to be feared. However, no actual abscess development occurred. The patients were miserable and protested strongly against the injections. Intravenous injection was then resorted to and proved to be the ideal method. There was no pain and no local reaction; whatever reaction occurred could be definitely attributed to the dose given, and the constitutional symptoms due to absorption from a focal area could be eliminated.

The initial dose was 1 cc. The reaction from this was observed and the next dose was gauged accordingly. In children the dose should be proportioned according to size and age. Some of the cases were started on 0.5 cc. doses, which were increased according to the report of the patient with regard to the effect of the injection upon the general condition. One cubic centimetre was the maximum intravenous dose. The injection was never given oftener than once a week, and as the case progressed the time between doses was lengthened to two, three and four weeks, as determined by physical examination, sputum examination and the needs of the individual.

Clinical judgment was based on increase or diminution of infiltration as determined by the whispered voice sound, the amount of moisture in the lungs as indicated by the character of the râles, increase or diminution in the respiratory rate, increase in the pulse rate and the general condition of the patient. Fever of one or two degrees was not considered a contraindication. The number of bacilli appearing in twenty microscopic fields at the beginning of the treatment was recorded, so that at subsequent examinations, with the same technique, a fairly accurate comparison could be drawn. The sputum was examined at monthly intervals.

Through this system of analysis it was possible to observe the effect of each injection and record the physiological action of the ethyl esters. The difference between this method and that adopted by others will at once become apparent when this phase of the treatment is discussed.

The intravenous injection of 1 cc. of ethyl esters of chaulmoogra oil has its primary effect upon the cough, which may be immediately increased. A tickling sensation in the larynx, one or two hours later, may start a paroxysm of coughing which is accompanied by increased expectoration. The cough will gradually subside, so that within twenty-four hours both of these symptoms will have returned to normal. During this time the volume of sputum will have increased from a third to a half. After three or four injections there is a noticeable decrease in the amount of sputum, a marked change in character of cough, and a diminution in the paroxysms of cough after each subsequent injection.

In laryngeal tuberculosis these symptoms are greatly aggravated, but are followed very rapidly by a general feeling of comfort and an improvement which is in strong contrast to the previous clinical picture. The lung shows an increase in the area of infiltration, which is coincident with the cough, and which persists for from two to three days following

the injection, and then gradually clears. There is a definite increase in the amount of moisture detected in the lung which also disappears with the recession of the reaction. There was never any aggravation of the bleeding in any of the hemorrhage cases, neither was there evidence of any cumulative action in the lung, as reported by Walker in his guinea pig experiments.

The action on the heart is variable. Some of the patients made no comment about heart symptoms. Others complained of precordial distress accompanied by dyspnoea. Examination of the heart did not show sufficient clinical evidence to explain the discomfort. One patient complained of vertigo, precordial distress, a tendency to faint and an inability to walk five minutes after a dose of 1 cc. Her heart was regular, with a rate of 80, and she had no objective evidence to account for the subjective manifestations. These symptoms lasted for two hours. On the day after the injection the patient had an urticaria limited to the arms and legs, evidently an anaphylactic reaction, the only one in all the treatments given. After several injections, between four and eight in some cases, there is evidence of a cumulative action on the heart. This is recognized by increase in the pulse rate, a forcible precordial impulse, increased respiratory rate and changes in the auscultatory findings. The first sound is more booming in character and is decidedly accentuated over the second sound. The entire heart picture gives the impression that there is an actual increase in the heart labor. This reaction must be remembered in treating hemorrhage cases. No striking blood pressure changes were noted.

The effect on the gastrointestinal tract is probably the most remarkable. In some instances there was no reaction at the beginning, but as the treatment progressed the patients complained of slight discomfort in the epigastrium accompanied by belching. These symptoms gradually became aggravated, until they were followed by nausea and vomiting, which persisted at intervals of twenty-four to forty-eight hours. There was also a decided decrease in the appetite during this active period. After the height of the irritation had passed the appetite returned and the patients had no further discomfort. When this stage was reached in the treatment, the dose was reduced to 0.5 cc., and, if this amount caused any gastric discomfort whatever, the next dose was withheld for from two to three weeks, the amount given depending upon the return of appetite and the general condition of the patient.

The nervous system was not the cause of any great concern. Other than slight headache, no symptoms of any consequence were recorded.

One case developed a kidney complication in the form of hemorrhage. There were no prodromes, but the urine contained blood twenty-four hours after the injection. The temperature rose but one degree above normal and the patient did not feel sick. Blood was present in gradually decreasing amounts for seven days until it finally cleared. It did not recur as the result of subsequent injection.

Two cases had a recurrence of the menstrual flow, one three and the other seven days after menstruation had ceased. In both cases the bleeding was scant and lasted intermittently for twenty-four hours. There was no repetition during the further treatment. One case had a mild epistaxis. One patient reported the loss of erectile power, with cramp-like pains in the muscles of the legs and a rigidity of the entire body.

The fever curve followed the other constitutional effects very closely. Those patients who had the most marked clinical reactions also developed the strongest febrile response. The height of the fever was never alarming, however, the highest rise noted being two degrees above that previously registered. In most cases it dropped, with the recession of the other symptoms, within the twenty-four or forty-eight hour period. Occasionally it would last over a period of from four to seven days, and return to its original point by lysis. The greatest temperature response was obtained in those patients already in the febrile stage. The patients who had no febrile reaction due to the disease when the treatment was started remained afebrile.

An analysis of the value of the treatment of tuberculosis with the ethyl esters of chaulmoogra oil, as observed in my cases, can best be accomplished by comparison with the experience of other investigators.

Walker and Sweeney (1) found that the sodium salts of the total fatty acids of chaulmoogra oil, incorporated in fluid culture mediums, were bactericidal in dilutions of 1 to 100,000 and antiseptic in dilutions of 1 to 1,000,000.

Rogers (2) (3) suggested its use in tuberculosis, but believed that intravenous injections might produce exacerbations because any softening of a tuberculous lesion, such as sometimes occurred in leprous nodules, might possibly lead to dangerous dissemination of the bacilli.

In a personal communication from Dr. Walter Alvarez I have learned that Walker in recent animal experiments, as yet unpublished, has shown

that the animals cannot stand more than a certain amount of the drug before their lungs become markedly hypertrophied, and that rabbits die after a certain amount becomes deposited in the lungs. Previous to this Culpepper and Albeson recorded distinctly retarding effects of soluble preparations of chaulmoogra oil on tuberculosis in guinea pigs, and Walker had obtained retardation, retrogressive effects and occasional complete disappearance of tuberculosis in these animals.

These experiments confirm only in part my clinical experience. The cumulative effect of the drug is not manifested upon the lung, as is evidenced by the improvement in cough, the change in amount and character of the sputum and the reduction of the bacilli in a given number of microscopic fields. It is more evident upon the heart and stomach. I am therefore of the opinion that the intravenous administration of the oil is the method of choice. Harper (4), in the treatment of over 200 cases of leprosy, has given over 26,000 intravenous injections without ill effects. My experience in tuberculosis coincides with his in leprosy that patients vary considerably in the dose tolerated and that the dose must therefore be carefully adjusted to each patient. I again wish to warn against the danger of increasing the dose and a routine treatment. I would advise the careful selection of the dose and its gradual diminution in the presence of improvement or failure.

In a study of 60 cases of tuberculous laryngitis, Lukens (5), using local application of the oil, concludes that it is of value for the relief of pain and dysphagia, and that improvement, while not all that could be desired, seems more encouraging than that obtained with other drugs. Philip King Brown considers the ethyl esters, applied straight, the most effective remedy for tuberculous laryngitis. In my cases two were complicated with tuberculous laryngitis, and both improved under the intravenous medication without local application.

These observations were based upon the treatment of 25 cases, for a period of three months to one year. The youngest patient was a girl fifteen years old, the oldest a man of sixty-five years. There were three hemorrhage cases, two cases of laryngeal tuberculosis and one case of suppurating tuberculous adenitis. All the others were uncomplicated pulmonary cases.

In comparing the intravenous with the intramuscular method, it must at once be evident that, where a decided local tissue reaction occurs after each injection, constitutional reactions, in proportion to the amount

injected, must follow. I have no doubt that the dose given by others was too large. In some instances from 1 to 3 cc. was used intramuscularly twice a week. This fact will account, in large measure, for many of the discouraging experiences which have caused the treatment to fall into disrepute at this time.

In my cases, some of which showed improvement in the cough, a lessening of the sputum with a decided diminution in the number of bacilli, and a decrease in the lung infiltration with a general increase in strength, the improvement must be credited to the ethyl esters of chaulmoogra oil. In others the betterment in the condition was not as striking. In general, however, I am of the opinion that doses of from 2 to 4 minims will be better tolerated than larger doses, and will not cause reactions when given at weekly intervals; that the dose should not be increased above 0.5 cc. in any case; and that when the maximum dose is used the interval between injections should be from two to three weeks. In any case the individual reaction must be the guide for further treatment. If these precautions are followed, I think that the further use of chaulmoogra oil esters is warranted in the treatment of pulmonary tuberculosis and its complications.

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THE SUSPENSION STABILITY RATE OF ERYTHROCYTES IN PULMONARY TUBERCULOSIS

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Fahraeus (1), in a monograph on the suspension stability of the blood, has shown that it is subjected to considerable physiological variations. Different groups of individuals are distinguished by different values of sinking velocity. The classification of the groups is regulated by age, sex and physiological conditions. For example, the suspension stability is greater in the newborn than in adults. There is also a distinct difference between the sexes, as women generally have a considerably greater suspension stability than men. Especially is this true in pregnancy, when it is observed that during the greater part of the gestation period the suspension stability is reduced and that this reduction becomes apparent during the first months of pregnancy and attains its maximum at the close of this condition and remains so for the first two months after delivery.

The sedimentation of erythrocytes according to Fahraeus can be used as a diagnostic measure in determining the various stages in pregnancy, as well as to differentiate pregnancy from other conditions. This investigator has also observed that menstruation hastens the speed of sedimentation of the red blood cells. These phenomena have been attributed by Fahraeus to the fibrinogen content of the blood, and his observations have also been corroborated by Linzenmeier (2).

Further investigations by Fahraeus have shown that the hastening of sedimentation can also be noted in various pathological conditions, such as malignant tumors, acute and chronic infections and in high fevers. In cases of progressive paralysis, tabes and arteriosclerosis Plaut (3) found an increase in the speed of sedimentation of erythrocytes. Popper and Wagner (4) found an increase in cases of syphilis, and György (5) in infants over one month. Investigations on carcinoma and sarcoma cases by Löhr (6) also showed an increased speed. Abderhalden (7), on the contrary, has shown that in cases of icterus the suspension stability is diminished.

The results from these various observations indicate that the suspension stability of erythrocytes is not specific for any disease. Physiologically, it is increased during pregnancy and during the menstrual cycle; pathologically, an increase may be noted in tumors, infectious disease and high fevers.

Various factors may influence the speed and the degree of sedimentation of erythrocytes. Kürten (8) has shown that when cholesterin is added to human blood the speed of sedimentation is increased, and the reverse phenomenon is observed when lecithin is added to the blood; when the two substances are added together no effect is noted. Starlinger (9) has observed that the addition of kaolin or animal charcoal retards the sedimentation of corpuscles, while gelatine, agar, and gum acacia accelerate flocculation and sedimentation, and tuberculin inhibits the sedimentation considerably. Kok (10) has shown that local inflammation influences the speed of sedimentation and that a marked reduction can be observed within twenty-four hours, which reaches its maximum in four days and returns to normal after several weeks. Runnstrom and Schow (11) found that the average rate of sedimentation of erythrocytes was distinctly higher in the thyroidectomized than in the normal goat.

Westergren (12) has carried on a comprehensive research upon the sinking velocity of erythrocytes in tuberculosis of the lungs. He found the sinking speed abnormally high in the majority of cases with this disease and came to the conclusion that it is increased in all those cases in which, judging from clinical signs in other respects, we have reason to assume that the pathological process is active, and that the extent of this blood change stands in a decided parallelism to the degree of activity. For this reason Westergren considers that the stability reaction is an important aid for stating the prognosis of this disease.

The sedimentation test of red blood cells has been found to be an excellent aid in making the diagnosis in those cases in which tuberculosis of the lung has been improperly diagnosed on account of indeterminate examinations. Dreyfus and Hecht (13) have used the sedimentation reaction as an addition to the methods of diagnosis and prognosis of tuberculosis. Before one attempts to correlate the result of the sedimentation test with the clinical findings it is necessary to exclude the possibility of a latent syphilis or the other conditions enumerated, which may influence the reaction in the direction of an increased speed of sedimentation. Dreyfus and Hecht have also shown that the test runs parallel to

the chronicity of the disease and indicates that a destructive process is going on in the lungs. These observers have shown that in the earlier stages of a chronic pulmonary tuberculosis there is a slight increase in the rapidity of sedimentation and the cirrhotic processes give relatively high values, while in the exudative stages there are seemingly lower values in the speed of sedimentation. Tuberculosis with cavity formation shows a greater increase in the speed of sedimentation than do corresponding forms without cavity formation.

Gräfe (14), using the sedimentation test as a means of differential diagnosis between latent and active tuberculosis and in differentiation from normals and also from other diseases, has observed some very interesting results. In normal individuals he has noted that the speed of sedimentation was somewhat higher in women than in men. In the active incipient cases of tuberculosis the settling of erythrocytes was higher than in normal males and females. In doubtful cases, in which the sedimentation test was not of marked assistance in helping to make a diagnosis, a provocative test was performed with subcutaneous injections of 0.03 to 0.1 mgm. of old tuberculin. Gräfe observed that an increased sedimentation resulted in cases of incipient tuberculosis, but the test was normal in healed cases or in normal individuals. It is also of interest to note that these small doses of tuberculin influence the sedimentation at a time when they do not affect the temperature, the local or general processes. In marked cases of tuberculosis the injection of tuberculin causes an increase in the speed of sedimentation.

Katz (16) in determining the sedimentation speed in the various stages of pulmonary tuberculosis has attempted to correlate the relationship of the suspension stability with the pulse-temperature curve. His results show that the speed of sedimentation is in proportion with the pulse-temperature rate, that is, the higher the pulse and temperature the greater the speed of sedimentation. Starlinger (16) has observed that in those cases in which there is a great destruction of tissue cells, one observes a marked sedimentation reaction, which is especially pronounced in cases of malignant tumor, pneumonia, florid syphilis and pulmonary tuberculosis.

As a result of the numerous investigations on the suspension stability of erythrocytes in tuberculosis, we thought it advisable that a study along this line might be of some aid in diagnosis or prognosis of the case to be determined. The cases studied were from the wards of the City of

Chicago Municipal Tuberculosis Sanitarium, and ranged from incipient tuberculosis to far advanced pulmonary phthisis with cavity.

METHOD AND RESULT

Different methods have been employed by various investigators. Fahraeus uses a test tube, 17 cm. in length and 9 mm. inner diameter, and the tube is graduated in millimetres. Two cubic centimetres of a 2 per cent sodium citrate solution is poured into the test tube and the blood, collected from the median basilic vein, is allowed to flow into the tube until it reaches the 10 cm. mark. The ratio of the citrate solution to the blood is 1 to 4 and the height of the citrate-blood mixture 150 mm. The time when the blood is collected is noted and the tubes are turned up and down several times to insure thorough mixing of the contents. The tubes are then placed in a vertical position in a test tube rack and the readings determined after one, two, and twenty-four hours.

Because of the fact that this technique employs large quantitites of blood and possibly occasions an uncomfortable feeling on the part of the patient, Fahraeus (1) has simplified his own technique by using a micro-method, which consists of drawing up into a small graduated syringe a drop of blood. Before drawing up the blood the syringe is filled with sodium citrate solution to one-fifth of its volume. After mixing the blood and citrate, the syringe is hung up vertically, mouth downward, and a reading is taken after twenty minutes by the aid of a millimetre measure placed on the outside of the glass tube. This author does not recommend this micromethod for the reason that the blood column does not become sufficiently high in the syringe.

Westergren's (12) modification consists of collecting the blood and citrate in a 1 cc. syringe, and the citrated blood is transferred into a pipette having an internal diameter of 2.5 mm. The advantage of this method is that a high blood column of about 200 mm. is obtained with a small amount of blood. This technique is also advantageous in that one can perform daily examinations with this method.

The method described by Katz is similar to that of Westergren. Starlinger uses 0.2 cc. of a 5 per cent sodium citrate solution in determining the sedimentation of red cells. Gräfe uses 1 cc. test tubes. The tubes used by Löhr were 6.5 cm. high and 5 mm. in diameter, graduated at 6, 12 and 18 mm.

Other methods have been used, such as mixing blood corpuscles from defibrinated and centrifugated blood with suspension fluid (F. Raue (17)) and the rapidity of sedimentation expressed in millimetres of fluid free from corpuscles. Abderhalden proposed centrifugating out the cells and testing the sedimentation time in a heterologous plasma, by which method he says that important information can be gained as to the agglutinating power of such plasma.

We have employed the original technique described by Fahraeus because in simplicity this method conforms with all the desired demands. This method is proposed for the clinician, for the results in all cases agree with those of the other methods described. This technique also permits the reading of the results to be made with ease.

The principle of the method has simply been that of measuring the velocity at which the red corpuscles descend in blood samples which have been prevented from coagulating by sodium citrate. In order to measure the sinking speed of the red cells, the distance which these cells descend in the test tube must be determined in a certain time. By measuring the height of the clear plasma layer above the lower red column of red cells the sinking speed can be obtained. It is necessary, therefore, that a certain period of time be adapted for the readings to be taken in all cases. The time employed for the readings of the results in our experiments have been after one, two and twenty-four hours.[1]

We have examined over 400 patients in the various stages of pulmonary tuberculosis, and the following tables are only typical examples of the number studied. In healthy men the sinking values average 0.33 cm. per hour and in healthy women 0.74 cm. per hour. Any values above 0.9 cm. per hour for men and 1.2 cm. per hour for women must be regarded as pathological changes in the suspension stability of the blood. Examination of tables 1 and 2 show that an increased speed of sedimentation is present in pulmonary tuberculosis and that the sinking velocity is more pronounced in the early cases. We find that the marked differences in the speed of sedimentation can be observed in the first two-hour readings of the tables. At the end of one or even two hours the suspension stability is markedly increased in the far advanced cases. The speed of sedimentation between the early, moderately advanced and far advanced cases may not show any very marked changes at the

[1] This time has been fixed at one hour on the following grounds: after the lapse of this time the sedimentation has in every case proceeded so far that one gets readable results but is never finished, not even in the most rapidly settling tests. (Fahraeus.)

TABLE 1

The suspension stability rate of erythrocytes in the various stages of pulmonary tuberculosis in female patients

NUMBER	CLASSIFICATION	TEMPERATURE	PULSE RATE	SPUTUM	WASSERMANN	COMPLEMENT DEVIATION	SEDIMENTATION RATE		
							1 hour	2 hours	24 hours
	Turban	*Fahrenheit*	*per minute*				*cc.*	*cc.*	*cc.*
1	I	98[8]	88	0	0	++	0.8	1.2	3.5
2	I	99	110	0	0	+++	2.9	3.9	5.6
3	I	98[6]	86	0	0	++	0.4	1.1	3.3
4	I	98[6]	88	0	0	0	1.0	1.4	4.4
5	I	98[6]	86	0	0	0	0.7	2.0	4.1
6	I	98[8]	100	0	0	++	0.4	1.0	6.4
7	Ic	98[6]	86	0	0	+++	1.2	1.9	3.7
8	Ic	98[6]	88	0	0	0	1.2	2.9	4.1
9	Ic	99	90	0	0	0	1.9	3.3	5.5
10	II	99[8]	90	+	0	0	3.5	3.8	4.7
11	II	99[2]	90	+	0	0	1.4	1.9	4.1
12	II	99	86	+	0	0	1.9	2.5	4.1
13	II	98[8]	90	+	0	0	2.6	3.0	4.7
14	II	99	86	+	0	0	2.2	2.5	4.8
15	II	98[8]	93	0	0	+++	3.2	3.8	4.2
16	II	98[8]	90	0	0	0	3.2	4.0	5.7
17	II	98[8]	86	0	0	0	1.7	2.7	5.0
18	II	99[8]	105	+	0	0	3.9	5.2	6.0
19	II	98[8]	88	+	0	++	2.3	3.4	4.8
20	II	99	92	+	0	0	2.7	3.2	5.2
21	II	99	94	+	0	0	2.1	2.8	4.3
22	II	98[8]	100	0	0	0	0.8	1.7	3.5
23	II	99	90	+	0	0	2.0	3.3	5.3
24	II	98[8]	88	+	0	0	2.9	3.9	5.3
25	II	99	90	+	0	0	1.3	2.7	4.3
26	II	99	90	+	0	0	1.5	3.1	4.7
27	IIb	101[3]	100	+	0	0	1.6	2.8	4.4
28	IIb	98[8]	90	0	0	+++	1.7	3.0	5.1
29	IIc	100[2]	101	+	0	0	3.5	3.8	4.7
30	IIc	98[6]	96	0	0	0	5.4	6.1	6.5
31	IIc	100[8]	105	0	0	+++	5.3	5.9	6.4
32	IIc	99[8]	110	+	0	0	5.0	5.3	6.1
33	IIc	99[2]	90	+	0	0	2.5	5.5	6.0
34	IIc	99[2]	88	0	0	0	1.4	6.0	6.3
35	III	101	100	+	0	0	5.5	6.1	6.3
36	III	100[4]	104	+	0	0	4.9	5.5	6.3
37	III	99[6]	100	+	0	0	4.5	5.5	5.9
38	III	101	100	+	0	0	4.0	4.6	6.0
39	III	100[4]	96	+	0	0	3.8	4.2	6.2
40	III	98[8]	98	+	++++	++	6.0	6.6	7.1
41	III	100[4]	99[8]	0	0	0	5.8	6.1	6.3
42	III	99[8]	110	+	0	0	4.9	6.0	6.6
43	III	100	120	+	++++	0	4.9	6.0	6.6
44	III	100[4]	100	+	0	0	3.5	6.0	6.5
45	III	101	100	+	0	0	4.7	5.2	5.6
46	III	100[6]	98	+	0	0	4.5	6.5	6.9
47	III	98[8]	90	+	0	0	4.6	5.5	5.9
48	III	100[3]	99	+	0	0	4.4	5.3	5.7

end of twenty-four hours, but in the first two hours there are enough differences visible to warrant the classification of these cases into their respective orders.

TABLE 2

The suspension stability rate of erythrocytes in the various stages of pulmonary tuberculosis in male patients

NUMBER	CLASSIFICATION	TEMPERATURE	PULSE RATE	SPUTUM	WASSERMANN	COMPLEMENT DEVIATION	SEDIMENTATION RATE		
							1 hour	2 hours	24 hours
	Turban	*Fahrenheit*	*per minute*				*cc.*	*cc.*	*cc.*
1	I	98^{8}	88	0	0	0	0.8	1.9	4.2
2	I	98^{6}	90	0	++	+	1.2	2.1	3.7
3	I	98^{6}	88	0	0	0	1.5	2.2	4.5
4	I	98^{6}	84	0	0	0	0.9	1.4	3.3
5	I	98^{6}	82	0	0	0	0.2	0.8	2.6
6	Ib	98^{6}	84	0	0	0	1.2	2.2	3.8
7	Ib	98^{6}	90	0	0	0	1.0	2.4	4.4
8	Ic	98^{8}	88	0	0	0	1.3	2.1	6.0
9	II	99^{8}	88	+	0	0	1.7	2.9	4.6
10	II	98^{8}	96	+	++++	0	5.3	5.5	5.8
11	II	99	89	+	0	0	1.4	2.1	3.5
12	II	98^{8}	90	+	0	0	1.4	2.2	3.9
13	II	90	86	+	0	0	2.4	2.7	3.8
14	IIc	99	90	+	0	0	2.1	2.5	4.0
15	IIc	99^{2}	92	+	0	0	2.3	3.6	5.2
16	IIc	99	90	+	0	0	1.5	3.5	5.5
17	IIc	99	90	+	0	0	4.3	4.6	5.0
18	IIc	99^{4}	92	+	0	0	3.0	3.4	4.3
19	III	100^{2}	96	+	++++	0	2.5	4.0	5.2
20	III	99^{4}	92	+	0	0	3.3	4.7	5.4
21	III	100^{4}	100	+	0	0	5.4	5.8	6.0
22	III	101	96	+	0	0	4.6	5.3	5.6
23	III	99^{3}	100	+	0	0	5.3	5.6	5.8
24	III	99^{4}	100	+	0	0	5.0	5.6	5.9
25	III	99^{6}	90	+	0	0	5.8	6.1	6.3
26	III	100	92	+	0	0	4.5	5.0	5.7
27	III	99^{4}	86	+	0	0	3.2	5.5	6.2
28	III	99^{2}	88	+	0	0	4.2	5.4	6.0
29	III	100	93	+	0	0	4.9	5.5	5.7
30	III	100	110	+	0	0	6.1	6.4	6.6
31	III	102	120	+	0	0	6.3	6.6	6.8
32	III	100^{4}	100	+	0	0	5.7	6.0	6.2

It is evident that the sinking speed is slightly increased in the female patients, whose blood, under normal conditions, will sediment more rapidly than male blood. Also, secondary diseased conditions will affect

the sedimentation reaction in the direction of an increased speed in the settling time (table 3).

In chart 1 the average speed of sedimentation in the various stages of pulmonary tuberculosis is shown. The black column represents the female patients, and the striated column the male patients. It will be observed that in the various stages of pulmonary tuberculosis the average sedimentation rate is slightly higher at the end of one, two and twenty-four hours in the female patients as contrasted with the male

TABLE 3

The suspension stability rate of erythrocytes in the various stages of pulmonary tuberculosis associated with secondary diseased conditions

NUMBER	CLASSIFICATION	TEMPERATURE	PULSE RATE	SPUTUM	WASSERMANN	COMPLEMENT DEVIATION	SEDIMENTATION			COMPLICATIONS
							1 hour	2 hours	24 hours	
	Turban	*Fahrenheit*	*per minute*				*cc.*	*cc.*	*cc.*	
1 ♂	III	102	110	+	0	0	4.5	5.7	6.0	Tuberculous ulcerations in intestinal tract
2 ♂	III	101	105	+	0	0	6.1	6.4	6.6	Tuberculous pleurisy. Sclerosis of coronary vessels
3 ♀	III	103	130	+	0	0	7.1	7.3	7.5	Purpura hemorrhagia
4 ♀	III	102[4]	120	+	0	0	6.0	6.2	6.9	Tuberculous laryngitis and tuberculous intestines
5 ♀	III	101	110	+	0	0	6.1	6.4	7.6	Interstitial nephritis. Abscesses in lungs and kidneys
6 ♂	III	103	122	+	0	0	5.4	7.0	7.4	Mitral stenosis. Tuberculous spleen. Sclerosis of coronary vessels. Tuberculous enteritis.
7 ♀	III	100[4]	108	+	0	0	3.5	6.0	6.5	Organic cardiac lesion

The diagnosis of all these cases with the exception of no. 3 has been corroborated by autopsy findings.

patients. In tuberculosis associated with secondary diseased conditions the average rate of sedimentation at the end of one, two and twenty-four hours is higher than in the corresponding times of any of the other stages of tuberculosis not associated with other diseased conditions.

There appears to be a divided opinion as to the diagnostic and prognostic value of the suspension stability test in pulmonary tuberculosis. The mere fact that the reaction can be elicited in more than one condition corroborates the assumption that this reaction is not specific. This

test might be of aid when it is used in association with other findings of the case. If an individual complains of an ailment which, clinically, might be pulmonary tuberculosis, and syphilis, tumor and pregnancy can be ruled out, the sedimentation reaction might be of extreme usefulness in helping to eliminate other conditions which we know do not give an increased sinking speed of red cells. When once the diagnosis of pulmonary tuberculosis becomes definitely established, the suspension stability is an important aid in helping to classify the condition into the stage in which it belongs. If the patient is kept under observation for some time and repeated examinations are made, this test is of value in

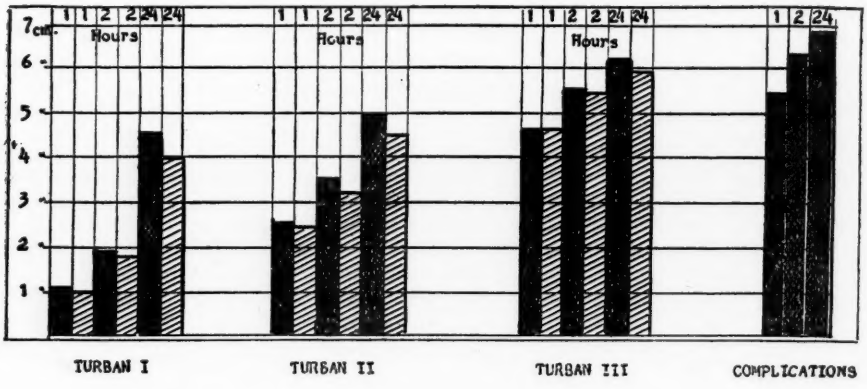

CHART 1. AVERAGE SEDIMENTATION RATES OF ERYTHROCYTES IN THE VARIOUS STAGES OF PULMONARY TUBERCULOSIS

Solid Column: Female patients
Shaded Column: Male patients
Complications: Include male and female patients

helping to determine whether the patient's condition is becoming better or worse. This, of course, can be determined if the degree of sedimentation becomes increased, a circumstance which is indicative of the breaking down of more tissue and a more extensive involvement of the diseased area. A decrease in the speed of sedimentation would indicate a possibility of the process becoming arrested.

In like manner this test can be used as an aid in prognosis. The rapid settling down of red cells within the first ten minutes to one hour would suggest a poor prognosis.

DISCUSSION

As the increase in the speed of sedimentation of erythrocytes is associated with changes in the blood plasma, numerous factors have been enumerated by different investigators to explain the phenomenon, but it is evident that fibrinogen appears to play an active rôle. To begin with, a *physiological* increase over normal in the percentage of fibrinogen occurs in one condition, namely, pregnancy. Dochez (18), Krösing (19) and Halliburton (20), among others, have shown that the fibrinogen content may be increased in many infectious diseases, such as pneumonia, pulmonary tuberculosis, peritonitis, etc. Furthermore, it has been demonstrated that the fibrinogen fraction is increased in those conditions in which we know or may expect that the sinking velocity of the red blood corpuscles is increased. This fact appears evident when it is observed that in the newborn the fibrinogen fraction is low (21) and the sinking velocity is also decreased, while in pregnancy the fibrinogen fraction is high and the sinking velocity is also increased (Fahraeus).

Our results show that in tuberculosis, which is associated with a high fibrinogen content (Levinson (22)), the suspension stability of erythrocytes is also increased. The sinking velocity of the red cells is greater in the far advanced cases of pulmonary tuberculosis. The moderately advanced cases also show an increased sedimentation, but this does not occur so rapidly in the first hour of the settling time as it does in the far advanced cases. The early or incipient cases also show that the red cells settle out very rapidly, and this is especially pronounced when blood collected from these types of cases is compared with normal blood. It may also be of interest to note here that cavity cases show a greater increase in rapidity of sedimentation than do corresponding forms without cavity.

The question of the influence of secondary diseases on the tuberculosis and their effect on sedimentation is of interest. We have demonstrated by our findings that an intercurrent disease, such as influenza or purpura hemorrhagica, influences the sedimentation test in the same way that an acute exacerbation of the tuberculosis would. An acute flaring up of a tuberculosis, whether it occurs in a quiescent or an active tuberculosis, entails a greater destruction of tissue, the latter depending on the stage of the disease. This condition corresponds to what one observes in chronic pulmonary tuberculosis with cavity formation. The probable explanation of this phenomenon might be found in the idea put forward by Frisch (23).

Frisch[2] has demonstrated that the breaking down of tissue results in the liberation of tissue enyzmes, which in turn play an important rôle in the formation of fibrin. The quantitative increase in fibrinogen can be observed in those conditions where the breaking down of tissue cells has been in excess of repair. Sakamoto (24) has shown that the extract of broken-down lung tissue has a greater effect on the coagulating mechanism of the blood than extracts of other tissues. Foster and Whipple (25) are of the opinion that tissue injury and inflammation exert a powerful stimulus on fibrinogen production and cause a prompt and notable increase in fibrin values. Mills (26) has demonstrated that the lungs yield the most potent product, which is capable of activating fibrinogen formation more than brain extract. These factors all lead to the assumption that whenever there is tissue destruction, and more so when there is breaking down of lung tissue, there usually results a quantitative increase in fibrinogen.[3] Since it has been demonstrated that the increased sedimentation is more pronounced in the active and far advanced cases of pulmonary tuberculosis and the latter is coupled with an increased tissue destruction which is associated with a quantitative increase in fibrinogen, it appears highly probable that this latter product may be an important factor in the mechanism of an increased speed of sedimentation of erythrocytes.[4]

There are other factors which also play a rôle in the speed of sedimentation of erythrocytes. Alderhalden calls attention to not only the composition of the blood plasma but also to the red cells themselves, in determining the sedimentation time of erythrocytes. Marloff's (27) experiments have shown that the size and hemoglobin content of the erythrocytes have a certain effect on their sedimentation time. Löhr (28) is of the opinion that the phenomena of electrical charging of the red cells themselves are responsible for changes in the rapidity of their sedimentation. Plaut believes that auto-agglutination, which depends on the fibrinogen content of the serum, is the possible cause for the sedi-

[2] Jene Formen der Tuberkulose, bei denen es zu entzündlichen Prozessen insbesondere mit starkerer Exudation und zu käsigen Zerfall und Gewebsdestruction kommt, einen erhöhten Fibrinogengehalt aufweisen im Gegensatz zu zirrhotischen Formen (Frisch, *Zeitschr. f. Tuberk.*, 1922, xxxvi, 360).

[3] Die Vermehrung des Fibrinogengehaltes scheint begründet durch vermehrten Gewebszerfall im Körper (Katz, *Zeitschr. f. Tuberk.*, 1922, xxxvi, no. 6).

[4] Die Senkungsbeschleunigung der Erythrocyten zum wesentlichen Teil auf einer Veränderung des Blutplasmas beruht, die ihrerseits wiederum von dem Grade des Zellzerfalls abhängig ist (Sadlon, P., *Klin. Wchnschr.*, 1922, i, no. 40, 1997).

mentation reaction. Leendertz (29) has noted a more rapid sedimentation in cases of infectious disease and tumors, and believes that this phenomenon may have some relationship with antibodies present in the blood. This author believes that immune bodies in the blood plasma modify the physicochemical conditions in a way which results in an acceleration of the sedimentation process, for the reason that the suspension stability is affected by the presence of these immune bodies.

The presence of colloidal particles in the blood may affect the speed of sedimentation. Fibrinogen markedly increases the rate. Globulin increases the rate, but albumin decreases it (Sakae and Tsutsumi (30)). Von Oettingen (31) has observed that an increased velocity of sedimentation is associated with changes in the plasma, and, aside from the quantitative differences of the various albumin fractions, there are other variable peculiarities of the physicochemical structures of the blood plasma which must also be taken into consideration. Durand (32) has noted that a marked difference exists in the proportions of globulin and of serum albumin in different groups of tuberculous patients. It is evident, according to the results shown by this investigator, that there is usually an augmentation of proteins in the serum of these patients. In very severe cases with cachexia the total content in proteins decreases to an almost subnormal proportion. In pulmonary tuberculosis the albumin-globulin relation is variable, and sometimes reversed through a considerable increase of globulin. The increase in globulin is independent of the increase in proteins and is proportionate to the extent and activity of the specific lesion. The partial variation in the proteins of the serum is not specific for tuberculosis and therefore has no diagnostic value; the quantitative variations in the globulin make it possible to form conclusions as to the activity of the tuberculous process. Since the varying rapidity of sedimentation is due to the variation in stability of the plasma, in which fibrinogen is the most labile component, it appears evident that there are factors aside from those enumerated that may influence the sinking speed of the red cells.

Sachs (33) has shown that various silver preparations may cause changes in dispersibility in the body, and thus affect the sedimentation time. Other drugs, such as adrenalin or pilocarpin, cause a quickening of sedimentation and this may possibly be due to an increase of the agglutination titre. Incubation of citrated plasma or the addition of calcium chloride to citrated plasma affects the sedimentation time. Tuberculin, as has been pointed out above, inhibits the sedimentation

time considerably, and Starlinger (34) believes that the constituents of the plasma and the tuberculin combine to form a larger complex, in which the reaction is not specific for the plasmas of tuberculous patients. Also, the effect of different electrolytes and non-electrolytes in the rapidity of sedimentation was tested out by Raue (17). His results show that, as the inhibiting action of electrolytes on the rapidity of sedimentation of red blood cells is parallel with their swelling action on the colloidal albumin bodies, there must be one component in them that is responsible both for their action on the sedimentation of erythrocytes and on the swelling of the limiting membrane of the protoplasm.

It appears evident from what has been pointed out above that, regardless of how the other contributing factors may influence the sedimentation test, fibrinogen may play the active rôle. In the very early cases of pulmonary tuberculosis there is a slight increase in the rapidity of the sedimentation time. This indicates a slight degree of tissue destruction, and hence a slight increase in the quantitative amount of fibrinogen over normal, which becomes manifest by the slight increase in the rapidity of sedimentation. As the process of destruction continues, the amount of fibrinogen in the blood becomes correspondingly increased, and the suspension stability is also correspondingly increased. The cirrhotic processes of pulmonary tuberculosis give relatively high values for sedimentation time. Repeated observations with the sedimentation test in tuberculosis may be a means of determining (by an increase in the sedimentation time) whether or not the diseased area is spreading or the involved area is becoming more or less arrested and the sedimentation time is therefore lessened.

CONCLUSIONS

It is evident from the data presented above that the sedimentation reaction is a useful and simple aid to the methods of diagnosis and prognosis of tuberculosis. It seems to parallel the degree of anatomic involvement of the lung.

The suspension stability test throws light on the activity and progressive nature of the tuberculous process.

Since the suspension stability test of erythrocytes depends somewhat on the fibrinogen content of the blood, this test in turn may be used as a gauge of the cell destruction going on, as measured by the corresponding amount of albumin disintegration.

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THE LIPOIDS AND ENZYMES OF THE BLOOD IN PULMONARY TUBERCULOSIS

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At various times studies have been reported dealing with alterations in the serum constituents in various diseases; more recently particular attention has been directed along these lines in tuberculosis. Among the serum constituents studied, antibodies, enzymes, blood lipoids, etc., may be mentioned; but, as a rule, there has been no correlation made in these studies, either as to these constituents themselves or as to the clinical manifestations of the disease in question.

Russian observers have made a special study of the lipases (Sieber); and more recently Frisch (1) has published studies in this direction. As far as we are aware, serum ereptase has not been studied. A number of analyses, however, have been recorded on the lipoid content of the blood in tuberculosis, and also a large number are available dealing with complement fixation and precipitin tests. The literature on the latter analyses is extensive and we need only mention the more recent reports of Corper (2), Brown and Petroff (3), Bronfenbrenner and Schlesinger (4) and Petroff and Nishida (5).

Bloor (6) has shown that when one of the blood lipoids is increased over normal similarly high values are to be expected for the other lipoids, and that there appears to be a balance between the lecithin and cholesterol in the blood. The increase of the lipoids over the normal indicates that changes associated with fundamentally altered active metabolic processes are taking place, and this has led Rudolf (7) to consider the lipoidal picture of diagnostic value. Especially is this of significance when it is remembered that in the therapy of tuberculosis a fat-rich diet has been used ever since phthisis has been known as a wasting disease. Pulmonary tuberculosis with protracted course is usually associated with toxemia and a rather marked degree of emaciation. This latter condition is usually associated with an alteration in the lipoids of the blood.

METHODS AND RESULTS

In the following study the effort was made to draw sufficient blood from the patient to carry out a complete analysis and to make the various tests that we had under consideration. The blood was obtained from patients of the Municipal Tuberculosis Sanitarium of Chicago. The clinical condition of the patient, as determined by the attending physician, was classified in the usual manner adopted by the hospital at the time that these examinations were made (1917). In the Turban-Gerhardt classification the incipient, moderately advanced and far advanced stages of pulmonary tuberculosis are subdivided as I A, B, C, II A, B, C, and III A, B, C; and we have purposely accepted this classification as sufficient for the purpose of the examination. The letters following the Roman numerals indicate the degree of involvement in the various stages of the disease.

The following examinations have been made and the method used in carrying out these tests are given:

Total fat was determined by the Bloor method (6).

Cholesterin was determined by the Bloor method.

Lecithin was determined by the Bloor method.

Complement deviation and precipitin reactions were graded as 1 to 4 plus, and the method used was that described by Corper and Sweany (8).

Lipase was determined by the ethyl butyrate method and titration was with phenolphthalein as an indicator.

Ereptase was determined by digestion of Witte peptone and titration for tryptophan by bromine water. The degree of the digestive reaction may be expressed from 1 to 4 plus.

Antitrypsin was determined by the method described by Jobling and Petersen (9).

In table 1 is shown the results of the serum examinations for 14 patients. It will be observed that, in general, an increase in the blood lipoids (fats) is noted in the early or incipient cases and the cholesterin values also show an increase in these cases. A marked diminution of these values is observed in those types of pulmonary tuberculosis associated with increasing severity of the disease. The lecithin content of the blood is somewhat less in the more severe cases than in the early cases of pulmonary tuberculosis. Neither the complement fixation nor the degree of precipitin reactions are parallel to each other, or to the

TABLE 1

The blood lipoids, lipases, complement deviation, precipitation, ereptase, and antiferment titre in the various stages of pulmonary tuberculosis

CASE NUMBER	CLASSIFICATION	TOTAL FAT	CHOLESTERIN	LECITHIN	COMPLEMENT DEVIATION	PRECIPITATION TEST	LIPASE	EREPTASE	ANTIFERMENT TITRE
		mg.	*mg.*	*mg.*					
128	ia	933	135	100	++++	++++	Low	High	Medium
61	ia	—	250	100	++	—	High	High	Normal
62	ia	793	300	300	—	—	Medium	Medium	Normal
64	ib	507	216	200	+	+	High	Medium	Normal
63	iia	513	321	100	+	+	—	—	—
67	iib	1060	322	100	+	+++	Low	Low	Medium
66	iiia	1240	400	100	++++	+++	High	Medium	Medium
110	iiic	373	48	70	0	0	0	Medium	High
113	iiic	520	57	90	+++	++	0	Medium	High
114	iiic	396	112	90	+	+++	0	Low	High
118	iiic	353	88	100	0	++	0	Low	High
126	iiic	343	81	80	++	+	0	Low	High
127	iiic	395	60	100	+	—	—	Low	High
160	iiic	560	156	100	+	—	—	Low	High

TABLE 2

Ereptase determinations in the various stages of pulmonary tuberculosis

CLASSIFICATION	NUMBER OF CASES STUDIED	LOW	MEDIUM	HIGH	PERCENTAGE WITH LOW TITRE
Turban		Titre	Titre	Titre	
I	20	6	10	4	30
II	15	7	4	4	46
III	14	8	5	1	57

TABLE 3

Lipase determinations in the various stages of pulmonary tuberculosis

CLASSIFICATION	NUMBER OF CASES STUDIED	LOW	MEDIUM	HIGH	PERCENTAGE WITH LOW TITRE
Turban		Titre	Titre	Titre	
I	18	3	20	5	16
II	13	3	7	3	23
III	14	13	1	0	90

TABLE 4

Antiferment determination in the various stages of pulmonary tuberculosis

CLASSIFICATION	NUMBER OF CASES STUDIED	LOW	MEDIUM	HIGH
Turban		Titre	Titre	Titre
I	11	3	5	3
II	11	0	2	9
III	14	0	0	14

clinical condition. On the other hand the enzyme, lipase, and ereptase titres are diminished in the more severe cases, while an increase in the antiferment titre is observed in the far advanced cases.

We have examined an additional number of cases for the ereptase (table 2), lipase (table 3) and antiferment (table 4) determinations. Examination of these tables shows that we again find a diminution of the lipolytic titre as well as the erepsin titre and an increase in the antiferment. This corresponds, in general, with the findings observed in other diseases and should not be considered as peculiar to tuberculosis.

DISCUSSION

In an investigation of the blood lipoids made by Frisch (1) he has shown that the fibrous and stationary cases of pulmonary tuberculosis show a high lipase serum titre while in the caseous, exudative, and cachectic processes the lipase titre was low. Further studies made by this investigator demonstrated that clinical improvement went hand in hand with a rise in the lipase titre and vice versa. In an examination of our results the same condition can be seen to take place.

The early cases of pulmonary tuberculosis show an increase in the blood lipoids, and, as the condition progresses toward the moderately advanced or advanced stages of pulmonary tuberculosis, the lipoids become correspondingly diminished. This observation has also been made by Pisuatschewsky (20) who has shown that the lipase was reduced in clinical cases of tuberculosis. Normally the lipase index is between 15 and 16, in the incipient cases 10 and 12, moderately advanced 6 and 8, and in the far advanced cases 3 and 4. Recently Epstein and Lande (19) have shown that wasting diseases other than hyperthyroidism are associated with a low blood cholesterol. The increased lipoids of the blood may possibly be due to the increased feeding in the early cases of pulmonary tuberculosis. Some of the increased lipoids may be derived from the destruction of cells. Then, as the pathological activity continues, an increase of the blood lipoids may result from the inability of the serum to get rid of the fat, as the low state of vital activity of the cells does not permit them to take up as much of the fat as a normal cell would.

According to Iscovesco (10) the lipoids are indispensable to the life and growth of the body. This author has shown that certain lipoids possess the power to influence certain organs, and that the lipoid iso-

lated from the liver can stimulate this organ and also increase the growth and weight of animals. Further researches have also demonstrated that the quantity of the lipoids contained in various organs is generally diminished when the organs are diseased. The lipoids afford a certain amount of protection against the rapid penetration of the cell by saponin bodies and bile salts (Boas (11)).

That the total lipoids and phosphates in tuberculosis are not in proproportion to each other, as they are in normal conditions, has been demonstrated by Grieniew (18). This author has shown that the cholesterin quantity may be increased in some cases of pulmonary tuberculosis and lowered in others. In all the organs, however, the lecithin formation is less than in the normal.

Shaw-MacKenzie (12) has shown that an increased production of lipases in the tissues is an important factor in the defensive mechanism of the body, especially in bacterial invasions; and this investigator advocates the use of any substance that will stimulate the formation of these bodies. While Shaw-MacKenzie believes that the lipases are of importance in immunity, this observation has not been demonstrated in tuberculosis. It is thought by some that the lipases may be the means of measuring the destruction of the waxy capsule of the tubercle bacillus, but Long (13) has noted that lipases and esterases are not concerned in the penetration and disruption of the waxy barrier of the tubercle bacillus. Long is of the opinion that lipolysis does not result in the splitting or solution of the wax itself, but a separation of the wax from its protein combination.

Bloor (6), Zucker (14) and Bang (15), among others, are of the opinion that lecithin is increased with other lipoidal changes in the blood during fat absorption. These investigators have not found an increase in cholesterol during fat absorption, while Iscovesco has noted some increase. The latter observer believes that this difference may be due to the fact that cholesterol appears late in the period of absorption and may be overlooked if repeated examinations are not made. In the absorption of large amounts of fat in the blood, lecithin becomes increased first and then cholesterol. It appears then that, in order to have one of these substances in excess, a large amount of lecithin and cholesterol must become absorbed. Bloor has further demonstrated that cholesterol and lecithin are antagonistic in many of their relations in the living body and it may be that the increase of cholesterol fol-

lowing that of lecithin is a reaction the purpose of which is to offset the injurious effects of an abnormally high concentration of lecithin.

There is also some evidence of a relationship between the cholesterol and fatty acid content of most tissues. Mayer and Schaeffer (16) have shown that inanition produces a rise in cholesterol in the tissues, especially in the muscle and liver and in the lipoid phosphorus which may be found in the lungs.

Some further interesting observations have been put forward by Bloor who has shown that

> The outflow of fat from the tissues into the blood may or may not cause an increase in the blood lipoids, depending on the balance between the inflow from the stores and the outflow to the tissues in which fat is being utilized. If inflow and outflow are equal, the blood lipoid level remains constant. If the inflow is greater than the outflow, due either to flooding the organism with fat beyond its capacity to deal with it as in the lipemia produced by bleeding, and also that occurring in the early days of fasting, or to diminished power of the tissues to remove fat from the blood as in diabetic lipemia, then the blood lipoids increase until sometimes a visible milkiness (lipemia) is produced. There may, however, be considerable increases in the lipoids before there is visible lipemia, and it cannot be assumed that the increases in this case are due to "soluble" lipoids such as lecithin alone, since cholesterol and fat may both be increased.

Great hemorrhage in certain animals (rabbits) produces an outflow of fat into the blood far greater than the normal mechanism can take care of, and the result is a lipemia. In other animals (dogs) it is apparently impossible to produce a lipemia by hemorrhage, probably for the reason that in these animals the ability to handle fat is much greater than in rabbits, which are not accustomed to much fat in their diet (Bloor).

Our findings in the present study of blood lipoids are in many respects similar to those enumerated above. The ability on the part of the human mechanism in pulmonary tuberculosis to store the lipoids probably becomes lessened as the disease progresses, probably due to the fact that human beings are not accustomed to much fat in their diet.

The complement deviation reaction or the precipitation reaction is no index as to the degree of tissue destruction and is not parallel with the findings of the lipases or lipoids. The observations of Henning (17) as to the cholesterol and lecithin content in the far advanced cases of pulmonary tuberculosis are similar to ours.

CONCLUSIONS

In the study of the blood lipoids in the various stages of pulmonary tuberculosis it was noted that, in general, an increase is observed in the early cases, while a diminution is associated with those types of pulmonary tuberculosis with an increasing severity of the disease.

The complement deviation and precipitation reactions are not parallel to each other or to the clinical condition.

The lipase, ereptase and enzyme titres are diminished in the more severe cases, while an increase in the antiferment is observed in the far advanced cases of pulmonary tuberculosis.

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THE CHEMISTRY OF TUBERCLE BACILLI[1]

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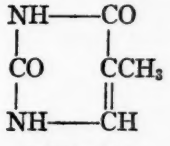
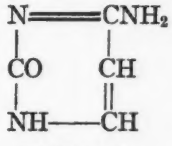
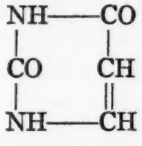
In an investigation of the chemistry of the tubercle bacillus which is being carried out in the Sterling Chemistry Laboratory the writers have finally succeeded in isolating the nucleic acid of this microörganism in sufficient quantity for an accurate determination of the pyrimidines contained therein. We find that its nucleic acid (tuberculinic acid) contains the two pyrimidines *thymine* (I) and *cytosine* (II). They were both isolated in a pure condition and in sufficient quantity for analysis. We were unable to detect the pyrimidine *uracil* (III); at least no evidence of the occurrence of this substance could be detected in the pyrimidine fraction of the hydrolyzed solution of the nucleic acid when tested by the delicate color test developed by Wheeler and Johnson (1).



I, Thymine



II, Cytosine



III, Uracil

The isolation and identification of thymine as a hydrolytic product of the nucleic acid present in tubercle bacilli confirms the assumption of Behring, Ruppel, Kitajima and Levene (2) that this pyrimidine functions as a part of the molecule, but these investigators did not definitely distinguish it from uracil. The isolation of cytosine from our nucleic acid is the first proof that this pyrimidine functions as a normal constituent of tuberculinic acid. Levene obtained, in the pyrimidine fraction resulting from hydrolysis of tubercle bacilli, a small quantity of a basic substance which he believed was cytosine, but the amount obtained was too small to permit of identification.

[1] The details of this study, including a description of the method of isolation of tuberculinic acid and the determination of the pyrimidines contained therein, are published in full in the *Journal of Biological Chemistry*, 1922, liv, 721.

This research was supported in part by a special grant from the National Tuberculosis Association for which we wish to express our thanks. We also wish to thank the Mulford Company and Parke, Davis & Co. for the material which made this investigation possible, and Dr. E. R. Long of the University of Chicago and Dr. Dorset of Washington, D. C., for samples of defatted and dried tubercle bacilli.

The fact that thymine and cytosine are found to be the only pyrimidines resulting from the hydrolysis of tuberculinic acid with 33 per cent sulphuric acid furnishes conclusive evidence that the conversion of cytosine into uracil does not take place as readily as is commonly believed. Levene performed his hydrolysis in an autoclave and at a higher temperature than that under which we operated. This very probably accounts for the mixture of pyrimidines which he apparently obtained. The results obtained by him on analysis indicated a mixture of uracil and thymine.

In our pyrimidine analyses the chief aim was to determine definitely which pyrimidines were actually present in tubercle bacilli and we therefore worked for a pure product rather than for the exact amount of each pyrimidine that could be obtained from our material. The results finally obtained indicate, however, that these pyrimidines, thymine I and cytosine II, are constituents of tuberculinic acid in about the same proportion. While we isolated somewhat larger amounts of cytosine than thymine, our results can probably be attributed to our method of operating, whereby we were able to avoid loss of material by mechanical procedure. If the assumption be true that tuberculinic acid contains the two pyrimidines in equal proportion, the finding of a larger amount of cytosine than thymine would be corroboratory evidence that cytosine was not converted into uracil under the conditions used for hydrolysis.

From table 1 it is evident that the pyrimidines which have been isolated from nucleic acids from different sources fall into two groups, namely, the nucleic acid from animal sources and the nucleic acid from plant sources. The pyrimidines characteristic of the nucleic acid from animal sources are thymine I and cytosine II, while the pyrimidines uracil III and cytosine II are found as constituents of plant nucleic acid. On this basis of classification the tubercle bacillus, which contains thymine and cytosine, is therefore definitely of the animal and not of the plant type.

Tuberculinic acid has been of special interest to biochemists since its discovery by Ruppel (3) who attributed to it great immunizing power. This property is no longer claimed for it, yet a knowledge of it is of interest from a chemical standpoint, as well as from the standpoint of classification of the tubercle bacillus phylogenetically as a plant or animal microörganism. Due to the scarcity of material available for investigation, as well as to the small amount of nucleic acid contained in tubercle bacilli, an accurate study of this phase of the work has not been made by earlier workers. A further study of this problem is now

being undertaken, and the writers hope with the amount of material at their disposal to be able to isolate tuberculinic acid in sufficient quantity to permit of very careful purification and analysis, and to determine accurately all of its constituents, thereby establishing the proportions in which this complex molecule is composed. It will be of interest to see whether tuberculinic acid is actually identical with other animal nucleic acids which have been submitted to careful examination. This work will finally require a very careful study of the nature of the sugar combinations which enter into the structure of this nucleic acid.

In the development of a practical method for the isolation of tuberculinic acid a large amount of preliminary work on tubercle bacilli was

TABLE 1

SOURCE OF NUCLEIC ACID	PYRIMIDINES
Animal nucleic acids	
Tubercle bacilli	Thymine and cytosine
Thymus gland	
Spleen	
Pancreas	
Liver	
Kidney	
Salmon sperm	
Shad sperm	
Sturgeon sperm	
Herring testes	
Ox testes	
Ox brain	
Human placenta	
Intestinal wall	
Plant nucleic acids:	
Yeast	Uracil and cytosine
Wheat (triticonucleic acid)	

done. In order to follow the progress of the work the mineral constituents of the material were first determined in both the human and bovine types which were used in our research. The distribution of phosphorus was carefully followed analytically in all the stages of separation of the components of tubercle bacilli. The exact percentages which were obtained are not intended to be taken to represent the exact amount of each fraction that might be obtained by a treatment, since we found the fractions to vary with the different types, yet the results obtained show in a general way the nature and distribution of the phosphorus-containing constituents which make up the tubercle bacillus.

We used toluene as the extracting solvent for the removal of the fat from the dry tubercle bacilli which required, for complete extracting,

20 parts of solvent to 1 part of the dried bacteria. Our procedure consisted in heating, at water bath temperature, with 3 parts of toluene for six to eight hours, and then allowing to stand over night. The toluene was then carefully decanted from the insoluble residue and the extraction repeated six times. After final treatment the residue was washed with boiling toluene until the washings were colorless. In this way practically fat-free material was obtained. The two units of tubercle bacilli furnished for our researches lost, on extraction with toluene, about 34 per cent and 43 per cent, respectively, of their weight. Analysis of the toluene extract and dried defatted bacteria revealed the interesting fact that the toluene extracts give an appreciable ash containing all the mineral constituents found in the original bacteria. This is shown in table 2.

The nature of the fat of tubercle bacilli has been studied more extensively than any other phase of the problem, yet the results obtained are far from conclusive. Quantitative methods for separation and identification of the organic products comprising such mixtures as that

TABLE 2

PRODUCT	ASH	P_2O_5 IN ASH	P_2O_5 IN PRODUCT	CaO IN ASH	MgO IN ASH
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Tubercle bacilli	4.19	47.88	2.01	5.75	7.72
Defatted residue	5.18	47.21	2.44	1.61	7.57
Toluene extract (fat)	2.27	53.75	1.23	24.64	8.82

represented by the material extracted by toluene from tubercle bacilli are not available; therefore, more work upon this particular phase of the chemistry of tubercle bacilli will have to be done before accurate chemical data can be presented. Considering the large amount of lipoidal material which we have obtained in defatting tubercle bacilli, further work along this line will be carried out along with our other work in the immediate future. Previous investigators have shown that the material extractable with fat solvents like toluene is a complex mixture containing many different kinds of constituents, such as fats, waxes, free fatty acids, alcohols, esters and phosphorus and nitrogen-containing compounds. The results of our analyses of the mineral constituents indicate that this mixture is even more complex than is generally thought. Most of the workers who have studied the fat of tubercle bacilli have not considered the mineral constituents present in this material. Goris reports an ash from the crude lipoid of 1.19 per cent, which he considered was made up largely of phosphates. While our analysis shows a P_2O_5 percentage consisting

of about 55 per cent, it also shows a calcium content of about 25 per cent (calculated as oxide) and an appreciable amount of magnesium. Since these constituents account for less than 90 per cent of the ash the remainder of the ash is probably made up of sodium and potassium combinations. An examination of the analytical data recorded in table 1 reveals a noticeable result in the large amount of calcium found in the toluene extract. When this value is compared with the percentage of calcium remaining in the residue it is observed that a much larger percentage of this element is found in the fat extract than in the defatted material. The percentages of the other mineral constituents are practically the same in the ash of the two fractions. Since the percentages of ash in the fractions differ considerably in amount, this fact must be considered in comparing the total amounts of the different constituents.

The defatted material remaining after the toluene extraction was next treated with hot alcohol, using 18 parts of 95 per cent alcohol to 1 part of dry material. The above extraction was carried out in three separate treatments, in which only a third of the alcohol was used at a time. The alcohol extract soon assumed a wine color, probably due to the atmospheric oxidation, apparently, of an extractable chromogen, and on cooling it deposited an appreciable quantity of precipitate. A precipitate was also produced upon diluting the alcohol extract with water. This behavior suggests that absolute alcohol would be a better solvent for this operation than 95 per cent alcohol. The alcohol removed 8.2 per cent of the weight of the defatted bacteria. Analysis showed that this material possessed an ash (6.69 per cent) larger than that found in the residue (5.31 per cent), of which above 80 per cent was accounted for as P_2O_5. The high phosphorus content of this material indicates the phosphatide nature of the constituents removed by alcohol.

The residue remaining after alcohol extraction gave a large ash (5.31 per cent) of which more than 40 per cent was composed of P_2O_5. The nitrogen content was also determined at this point and found to be 9.61 per cent. This material was odorless and was considered practically fat-free. It was used for hydrolysis with sodium hydroxide to remove the nucleic acid. After two treatments with caustic soda the material suffered a loss in weight of 16 per cent; however, only about 1.5 per cent of this was accounted for in the form of nucleic acid. The residue remaining after this treatment still contained a large amount of phosphorus (1.19 per cent), an amount larger than that removed by the treatment with alkali, although it contained no nucleic acid. A third treatment with caustic soda yielded no nucleic acid but only products

completely precipitated with acetic acid. The absence of nucleic acid in this residue was confirmed by a pyrimidine analysis, using the delicate color tests developed by Wheeler and Johnson (4) for the detection of uracil or cytosine, and Johnson and Baudisch (5) for the presence of thymine. No trace of either of these pyrimidines was found, showing conclusively that the large amount of phosphorus present in the residue after treatment with alkali did not represent a further quantity of nucleic acid.

The fact that the greater percentage of the phosporus contained in defatted tubercle bacilli still remains in the residue after the complete

TABLE 3

	GRAMS	PERCENTAGE OF TOTAL
Total P_2O_5 in 361 g. of tubercle bacilli (containing 2.01 per cent)	7.26	100
P_2O_5 removed by toluene	1.50	20.66
P_2O_5 removed by 95 per cent alcohol	1.12	15.42
P_2O_5 removed by NaOH (nucleic acid extraction)	2.57	35.39
P_2O_5 remaining in residue	2.17	29.89
Total P_2O_5 accounted for	7.36	101.36

removal of the nucleic acid shows the inaccuracy of the estimates of nucleic acid content of tubercle bacilli based upon the phosphorus content of this material. The estimates of the amount of nucleic acid contained in tubercle bacilli as a rule have been too high, due in part to the large phosphorus content of this material which was thought to be contained in nucleic acid combination. The same is true in regard to the protein residue which is usually regarded as consisting primarily of nucleoprotein, while the exact amounts of these two constituents in tubercle bacilli is as yet not definitely known. However, the estimates of Long, based on the amount of purines found, indicate that the nucleic acid content was not larger than 3.5 per cent, and that the nucleoprotein was not as large as commonly supposed, finds corroboration in our work.

Table 3 shows the distribution of phosphorus in tubercle bacilli calculated from the results obtained from human tubercle bacilli.

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SO CALLED SELECTIVE COLLAPSE IN ARTIFICIAL PNEUMOTHORAX

A PLEA FOR LOW INTRAPLEURAL PRESSURES[1]

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An interesting pneumodynamic phenomenon is frequently observed in the study of a certain group of cases with a free partial pneumothorax, and which, for want of a better term, has been characterized by some authors (1) as *selective collapse*. By this term is meant the tendency of the injected air to accumulate about the diseased part of the lung, *as if* selectively collapsing it, and leaving the healthy portions relatively less affected. The clinical significance of this phenomenon has been emphasized by some workers (1) (2) (3) in so far as it formed the theoretical basis for their practice of partial bilateral collapse. It seems to us, however, that this so called principle of selective collapse has a much wider clinical application in the management of pressure and in the study of reëxpansion at the completion of treatment. In order to evaluate properly its full clinical significance its mechanism must be thoroughly understood. It will, therefore, be the purpose of this communication (1) to analyze in some detail the mechanism of this phenomenon, (2) to indicate its clinical application in the management of pressures and (3) to show its value in the study of reëxpansion at the completion of the treatment.

THE MECHANISM OF THE PHENOMENON

If we induce a pneumothorax in an animal with normal lungs, we shall notice that all portions of the lung are collapsed fairly uniformly, the degree of collapse depending upon the amount of gas injected and the pressure achieved. As the air of the induced pneumothorax is gradually absorbed, the various portions of the lung will be seen to reëxpand equally and in proportion to the rate of gas absorption. The reason for this uniformity in the collapse and reëxpansion of the healthy lung

[1] From the Montefiore Hospital Bedford Sanatorium for Incipient Tuberculosis, Dr. M. Fishberg, Chief of Service.

is to be found in the uniform compressibility and elasticity of normal lung tissue.

On the other hand, in a case where a portion of the lung is diseased, induced pneumothorax will be seen to produce less uniform results. It is well known that among the earliest functional changes occurring in diseased lung tissue is a diminution in its compressibility and elasticity. And for this reasen it will be noted that the diseased portion of the lung will, on the one hand, be less compressible than the surrounding or adjoining lung tissue and, on the other hand, because of its diminished pulmonary elasticity, it will reëxpand less readily and completely than the adjacent normal lung, when the induced pneumothorax is slowly absorbed. In this connection, our experience with the difficulty in reëxpansion of injured portions of rabbit lungs after a short period of compression was quite illuminating. It was our repeated observation that, if any part of the rabbit's lung was severely injured during the attempt to induce a pneumothorax, that portion of the lung could not be made to reëxpand to any considerable degree, even after one week's compression, because of the inflammatory and fibrotic change engendered by the injury; at the same time, the uninjured parts of the lung under compression could be made to reëxpand to their full capacity by forced intratracheal inflation after its removal from the thorax.

In studying a case roentgenoscopically during the first few refills, it will be seen that the healthy portion of the lung is first to be compressed while the diseased portions "give" very slowly, thus allowing the bulk of gas to accumulate in the neighborhood of the healthy lung. But with repeated gas inflations the diseased portions of the lung will be observed to become more thoroughly compressed, while at the same time the tendency of the lung to reëxpand will be gradually reduced because of the progressively decreasing elasticity of the relaxed lung and particularly the tendency to rapid fibrosis occurring in inflamed tissue following surface approximation. The healthy portions of the lung, on the other hand, seem to retain their elasticity to a very marked degree, and their tendency to reëxpansion asserts itself just as rapidly and in proportion as the intrapleural pressure falls with the absorption of the induced pneumothorax.

These basic facts of physiological pathology rationally explain the tendency of the injected air to accumulate gradually about the diseased portion of the lung *as if* selectively compressing it, while leaving its healthy parts relatively less affected. There is nothing deliberate or

purposeful on the part of the air in this happy arrangement; hence our hesitancy in accepting the term selective collapse without qualification. The air in the pleural cavity will distribute itself in accordance with the particular dynamic conditions created there, intrathoracic equilibrium always being maintained.

As was already stated, these phenomena are best observed roentgenoscopically, and in suitable cases the entire mechanism can be studied in all its phases. We will endeavor to illustrate graphically the various points under discussion by a series of roentgenograms. Figures 1 and 2 represent the condition of the lungs in expiration and inspiration, respectively. These plates were taken three months after the induction of pneumothorax in a case with a lesion in the right upper lobe with relatively little or no involvement in the lower parts of the lung. It will be noted that during expiration (fig. 1) the upper lobe appears fairly well collapsed while the lower portions of the lung are but little compressed. During inspiration (fig. 2), on the other hand, the upper lobe seems to expand very little if at all, while the lower lobes are apparently able to expand to nearly their full capacity.

This state of affairs was achieved only gradually and after repeated inflations. Because of the extensive involvement of the upper lobe with a rigid cavity its compression was accomplished gradually, and even now the collapse is not absolute. At the same time, because of the relatively unimpaired compressibility of the healthy and healthier lower portions of the lung, the latter were most readily compressed. In other words, shortly after the first inflation most of the injected air was collected about the less involved and entirely healthy portions of the lung. But in course of time, because of the decreased and progressively decreasing elasticity and especially the increased tendency to fibrosis in the affected lung tissue under gradual compression, the involved upper lobe was able to reëxpand less and less as the intrapleural pressure fell following the absorption of the injected air, while at the same time the healthy portions of the lung below, the elastic recoil of which remained practically unimpaired even under compression, were able to reëxpand just as rapidly as the falling intrapleural pressure would allow it. It thus happens that after a longer or shorter period, depending upon the degree of involvement, the injected air accumulates about the involved portion of the lung *as if* selectively compressing it, leaving the healthier portions of the lung relatively little affected as to function.

That this state of affairs becomes more permanent in course of time is shown by the two plates, figures 3 and 4, taken in expiration and inspi-

ration respectively. These were taken one year after pneumothorax was induced because of an extensive involvement of the right upper lobe. Here it will be noted that, while collapse and cicatrization occurred in the upper lobe, the lower two lobes are relatively little affected and are able to function almost normally.

The natural tendency for atelectasis and fibrosis to develop in a tuberculous focus is greatly accentuated in the presence of pulmonary relaxation and surface approximation following the induction of a pneumothorax. This is true whether an entire lobe or only a small portion of it is involved; and whether the lesion is in the upper lobe or in any other part of the lung. So characteristic is this tendency that occasionally one can discover a previously unsuspected focus of disease by observing the changes occurring after the induction of a pneumothorax.

Figures 5 and 6, of plates which were taken in expiration and inspiration, respectively, well illustrate this point. These were taken about three months after the induction of pneumothorax in a case in which an active focus in the mesial portion of the right base was diagnosed. It will be noted that in figure 5 (expiration phase) the upper lobe, and more so the lower lobe, are considerably collapsed, while the middle lobe is only partly compressed. In figure 6, taken during the inspiration phase, the inner half of the lower lobe is seen to expand almost not at all, the upper lobe does so very little, while the middle lobe expands to a very much greater extent.

As already stated, the pneumothorax was induced in this case because of the diagnosed active focus in the inner half of the base. The focus in the upper lobe was questionable, judging from the roentgenological examination on admission. However, the behavior of the upper lobe under compression convinced us that it was more or less involved. We observed that its compressibility, as well as its tendency to reëxpansion, were somewhat diminished by comparison with the normal middle lobe, characteristics which were exhibited by the more involved lower lobe to a very much greater degree. In other words, we noted that during the early refills the relatively normal middle lobe was the first to be compressed, the slightly involved upper lobe yielded to compression less readily, while the extensively diseased mesial half of the lower lobe resisted compression to a marked extent. At the same time, following the absorption of the air between refills, the tendency to reëxpansion was observed to be most marked in the relatively unaffected middle lobe, less so in the somewhat involved upper lobe and

least in the mesial half of the lower lobe which was the seat of the active focus.

We recently had an unusual opportunity to observe the extreme delicacy of the mechanism of this phenomenon in a case in which pneumothorax was induced because of an active but circumscribed focus with cavitation in the inner portion of the right lower lobe. The readiness with which a marked compression of all lobes excepting the involved portion of the base was achieved was striking. The tendency to ready reëxpansion, following the gradual resorption of the injected air between refills, was also very remarkable. However, the point of greatest interest to us was the behavior of the affected mesial portion of the right lower lobe. This portion of the lung yielded to compression very slowly and after repeated refills only. In this case as in the other cases already described, the natural tendency to atelectasis and fibrosis in the tuberculous lower lobe was greatly increased by the compression, with its resulting pulmonary relaxation and approximation of inflamed surfaces, while at the same time its tendency to reëxpansion between refills decreased progressively. And thus it came about that in the course of seven months the involved portion of the base remained at nearly complete rest even during inspiration, thus allowing healing and fibrosis to proceed rapidly, while at the same time the uninvolved portions of the lung were able to function nearly or quite normally. We were able to study this case in detail roentgenoscopically; and though we have a series of roentgenograms illustrating graphically the various points brought out above, we are, for the sake of economy in space reproducing only two of these, which show respectively the condition of the treated lung before and after pneumothorax was induced. Figures 7 and 8 are self-explanatory and require no further elucidation. It is pertinent to add that the patient is at present free from local and constitutional symptoms.

The manner of localization of the injected air in the cases presented, accumulating as it does in the vicinity of the involved lung tissue wherever that may chance to be and in proportion to its degree of involvement, suggests a purposefulness or a guiding intelligence almost supernatural. However, the detailed analysis of the mechanism of the phenomenon has disclosed how this favorable distribution of the injected air is governed by physical laws and is determined by the particular pneumodynamic conditions of the individual case.

With this consideration of the mechanism of so called selective collapse before us, we may now proceed with the discussion of its clinical signifi-

cance in the management of pressures in induced pneumothorax, as well as its value in the study of reëxpansion of the lung at the completion of its treatment.

CLINICAL CONSIDERATIONS

The mode of action of induced pneumothorax, the manner in which it produces its beneficial effects, is still a disputed question. However, it is apparent that the mechanical factors of collapse must not be undervalued; compression of the involved lung tissue causes the evacuation of the fluid excretions and prevents their accumulation; it also renders the caseous areas drier and reduces the size of infected surfaces. But, from experimental and clinical evidence, it seems most probable that the beneficial effects of induced pneumothorax are due, directly and indirectly, to the fact that the already existing tendency to atelectasis and fibrosis in tuberculous lung tissue is greatly aided by the pulmonary relaxation and surface approximation resulting from compression. These changes induce lymphatic stasis and thus limit and may even prevent absorption of toxic products. This reduction of absorption releases the nervous system and all other organs from the chronic poisoning which is responsible for the constitutional symptoms; and it is the removal of the toxemia, and not the pulmonary collapse *per se*, which appears to be the important factor.

If, by suitable management of pressures, we are able to accomplish all the favorable results in the involved portions of the lung without at the same time seriously interfering with the functioning of its normal parts, should this not be our procedure of choice? In our analysis of the mechanism of so called selective collapse we emphasized the fact that these beneficial effects can and often do occur in the involved areas under a compression which is so slight as to allow a fair degree of functioning of the healthy portions of the lung. It seems to us, therefore, that the old teaching, to establish in all cases a complete collapse as early as possible and then endeavor to maintain it consistently by suitable positive pressure, is fallacious. This practice, which is recommended even by so eminent a clinician and experienced worker in the field of pneumothorax as Riviere, seems to us to be entirely unwarranted in the light of our better understanding of the pneumodynamics of induced pneumothorax.

In our studies on intrathoracic equilibrium in pneumothorax and its clinical significance (4) (5) we have shown that the most important

factor in the successful application of this mode of therapy is the determination and maintenance of an optimum intrapleural pressure in the individual case. By optimum pressure is meant the lowest pressure which is able to accomplish the necessary degree of compression with the least possible disturbance of intrathoracic equilibrium and consequent embarassment of the cardiorespiratory functions. While we fully realize the necessity and even urgency of very high positive pressures in cases with adhesions, a fixed mediastinum and, in the presence of definite indications (such as a tendency to pleural obliteration, very extensive disease throughout all lobes, and active thick-walled cavities), we warned most emphatically against the use of positive pressures in the presence of a labile mediastinum. Furthermore, our studies of the mechanism of the so called selective collapse show that the optimum pressure in most of such instances is very likely to be a moderate negative pressure which does not produce a complete collapse of the entire treated lung.

The disadvantages of complete collapse of the treated lung, the healthy and diseased portions alike, are too obvious to require detailed consideration. Suffice it to recall that the positive pressure requisite to establish and maintain a complete collapse causes considerable cardiovascular embarrassment as well as a displacement of mediastinal structures, with consequent interference with the function of the opposite untreated lung and resulting danger of reactivating quiescent foci which it may contain.

And to our mind one of the great objections to this procedure is that it is entirely unwarranted in most instances. As was brought out in the early part of this paper, all the good effects that are admittedly due to compression can be accomplished with pressures frequently so low as to interfere very little, if at all, with the normal function of the healthy parts of the lung. How this apparent selective collapse of the involved portions of the lung is brought about under these circumstances has been sufficiently discussed and illustrated so as not to require repetition.

Our experience during the past few years has convinced us that the cases in which pressures were managed according to the principles above described do at least as well as those in which complete collapse was induced. Barlow and Kramer seem to have had similar experience in their practice with more serious cases. These authors, in addition, have called attention to the few effusions developing in the course of treatment with partial compression. According to them the frequently developing effusions in the cases treated with positive pressures are due to the rupture of lung tissue at or near stretched pleural adhesions. Whether or not this explanation is entirely correct, it does appear to be a

fact that fewer effusions develop in cases with lower pressures and frequent refills than is the case when positive pressures and less frequent refills are used.

However, the two greatest advantages of this method are yet to be mentioned. First, with it induced pneumothorax can be employed in the treatment of a very much larger group of cases than would be possible were complete collapse to be used. The number of cases which may be considered suitable for this form of treatment is far greater because of the relatively little strain that is put on the healthy and less involved portions of the lung, on the treated as well as the untreated side, thus eliminating to a great extent the danger of reactivating latent foci. Second, and to our mind probably the greatest advantage of this method lies in the more perfect control we can exercise with it over the pneumothorax, especially with reference to the ultimate reëxpansion at the completion of the treatment. With this procedure of frequently repeated small inflations under optimum pressures, the compression and eventual collapse of the diseased portions of the lung are soon accomplished without at the same time seriously interfering with either function or elasticity of its uninvolved parts. And just as soon as we deem it advisable, we may allow the healthier portions of the lung to reëxpand entirely, while still being able to maintain the collapse of the diseased parts. Not infrequently, following the reëxpansion of the healthy parts of the lung pleural adhesions form and convert the free pneumothorax into one which is strictly localized about the most involved part, and the latter can be maintained long enough to achieve complete scarring. Under these circumstances even chest deformity may be avoided when all refills are stopped and the air of the localized pneumothorax is allowed to absorb; for the compensatory emphysema of the uninvolved parts of the lung will fill to a large extent the vacant part of the pleural space resulting from the shrinkage and fibrosis of the diseased portions of the lung, thus precluding undue displacement of mediastinal structures and falling in of chest wall. Figures 9 and 10 of plates taken one and a half and two years, respectively, after the induction of pneumothorax, and figure 11, a plate of another case in which the treatment is about to be terminated after four years, illustrate the points under consideration. Figures 9 and 10 show that the involved upper lobe in this case underwent a complete scarring, while the two healthy lower lobes have not only reëxpanded but are now undergoing compensatory emphysema in an effort to fill up the pleural cavity above as rapidly as the air of the pneumothorax there is being absorbed. Figure

11 shows an analogous state of affairs in the other case in which the treatment is about to be discontinued after four years.

When we contrast with this the state of affairs usually found in cases treated with complete collapse over a period of years, the striking advantages of the method advocated become apparent. In such cases there will occur, at the termination of treatment, great stretching of the compressed lung tissue which is likely to result in reactivation of dormant foci. Furthermore, there may also occur a great deal of mediastinal displacement and diaphragmatic distortion, as well as a falling in of the chest wall with consequent deformity. As an alternative to this, there remains the prospect of keeping up the refills perhaps for the rest of the patient's life, a procedure of choice in some of the cases, in order to avoid the dangers and discomforts of reëxpansion under such circumstances.

In conclusion, and lest we be misunderstood, we would emphasize the fact that we do not recommend the indiscriminate practice of partial compression in all cases treated with induced pneumothorax. Our plea is for the maintenance of an optimum pressure, that is, the least pressure capable of accomplishing the desired beneficial results without undue disturbance of the cardiorespiratory functions. As we pointed out elsewhere in this paper and in our earlier publications on the subject, we realize the necessity of even very high positive pressure in a certain group of cases with a fixed mediastinum and in the presence of definite indications (such as a tendency to pleural obliteration, extensive disease in all lobes of the treated lung, and active thick-walled cavities). But theoretical considerations as well as clinical experience have convinced us that in most cases with a flexible mediastinum the requisite optimum pressure to produce collapse of the involved parts of the lung may be so low as to produce only partial compression of its uninvolved portions. It is against the use of complete collapse in such cases, as a practice both unwarranted and dangerous, that we enter our most emphatic protest.

SUMMARY

1. By so called selective collapse is meant the tendency of the injected air to accumulate about the diseased part of the lung, *as if* selectively collapsing it, and leaving the healthy portions relatively unaffected.

2. This peculiar localization of the injected air is determined by the particular pneumodynamic conditions in the treated hemithorax:

a. The diminished pulmonary elasticity and increased tendency to fibrosis in the diseased portion of the lung under compression prevent ready reëxpansion of the latter.

b. At the same time, the elastic recoil of the healthy part of the lung remains relatively unimpaired, and its tendency to reëxpand asserts itself with each inspiratory effort and as the intrapleural pressure falls with the absorption of the injected air between refills.

c. Under these circumstances the air must localize to a great extent about the involved portion of the lung where it meets relatively little resistance to its accumulation.

3. Cases are presented and illustrated with roentgenograms to show the delicacy of the mechanism of this phenomenon under various circumstances.

4. In a large group of cases suitable for induced pneumothorax, the collapse of the involved portion of the lung, with all the resultant good effects that are admittedly due to compression, can be accomplished with pressures frequently so low as to interfere very little, if at all, with the function of the healthy parts of the lung.

5. While it is admitted that in a limited group of cases with a fixed mediastinum, and in the presence of certain definite indications, the use of even very high positive pressures may be justifiable, it is claimed that the old practice to establish and maintain in all cases a complete collapse of the treated lung by suitable positive pressures is entirely unwarranted in the light of our better understanding of the pneumodynamics of induced pneumothorax.

6. Among the great advantages of this method the following are particularly noteworthy:

a. The more perfect control over the pneumothorax that can be exercised with it, especially with reference to the ultimate reëxpansion of the lung at the termination of its treatment.

b. The possibility of avoiding chest deformity and the danger of reactivating dormant foci which are incident to excessive pulling of mediastinal structures and stretching of collapsed lung tissue, and which are likely to occur when complete collapse of the entire treated lung is resorted to.

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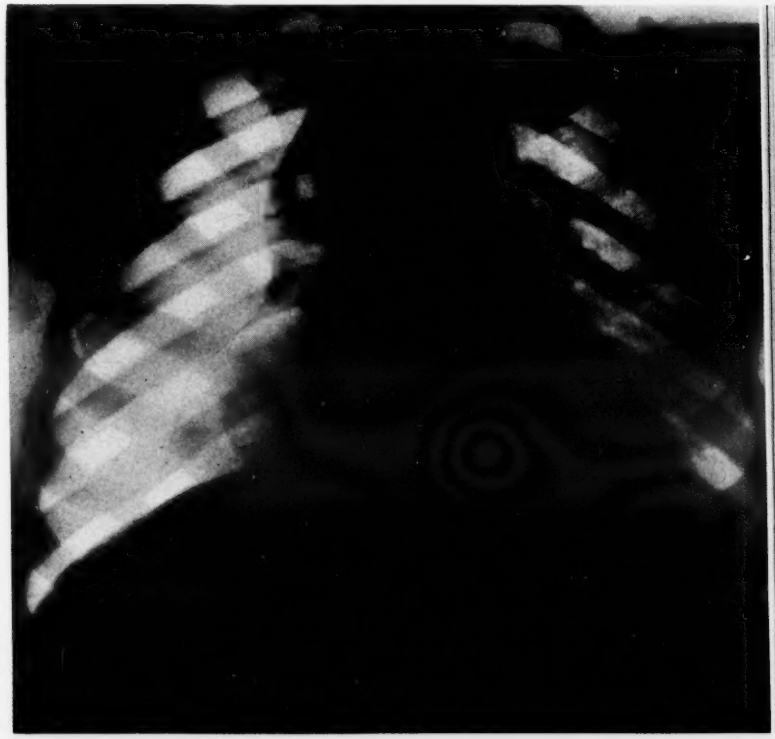

FIG. 1

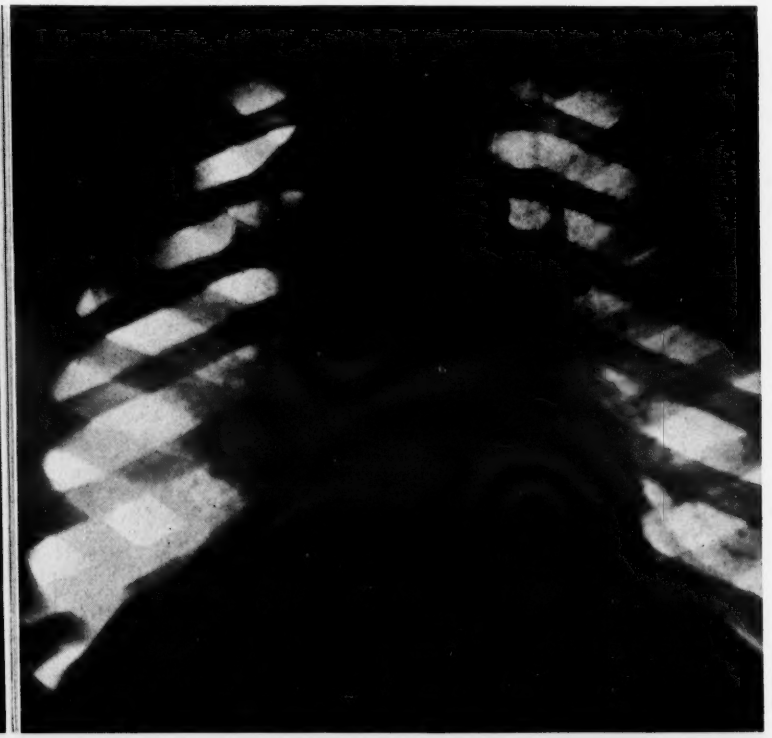

FIG. 2

Taken in expiration and inspiration, respectively, show the selective collapse of the involved right upper lobe and the moderate compression of the lower lobes. In figure 2 note how the uninvolved lower lobes are able to expand nearly to their full capacity during deep inspiration while the involved upper lobe expands little, if at all. Plates were taken three months after the induction of the pneumothorax.

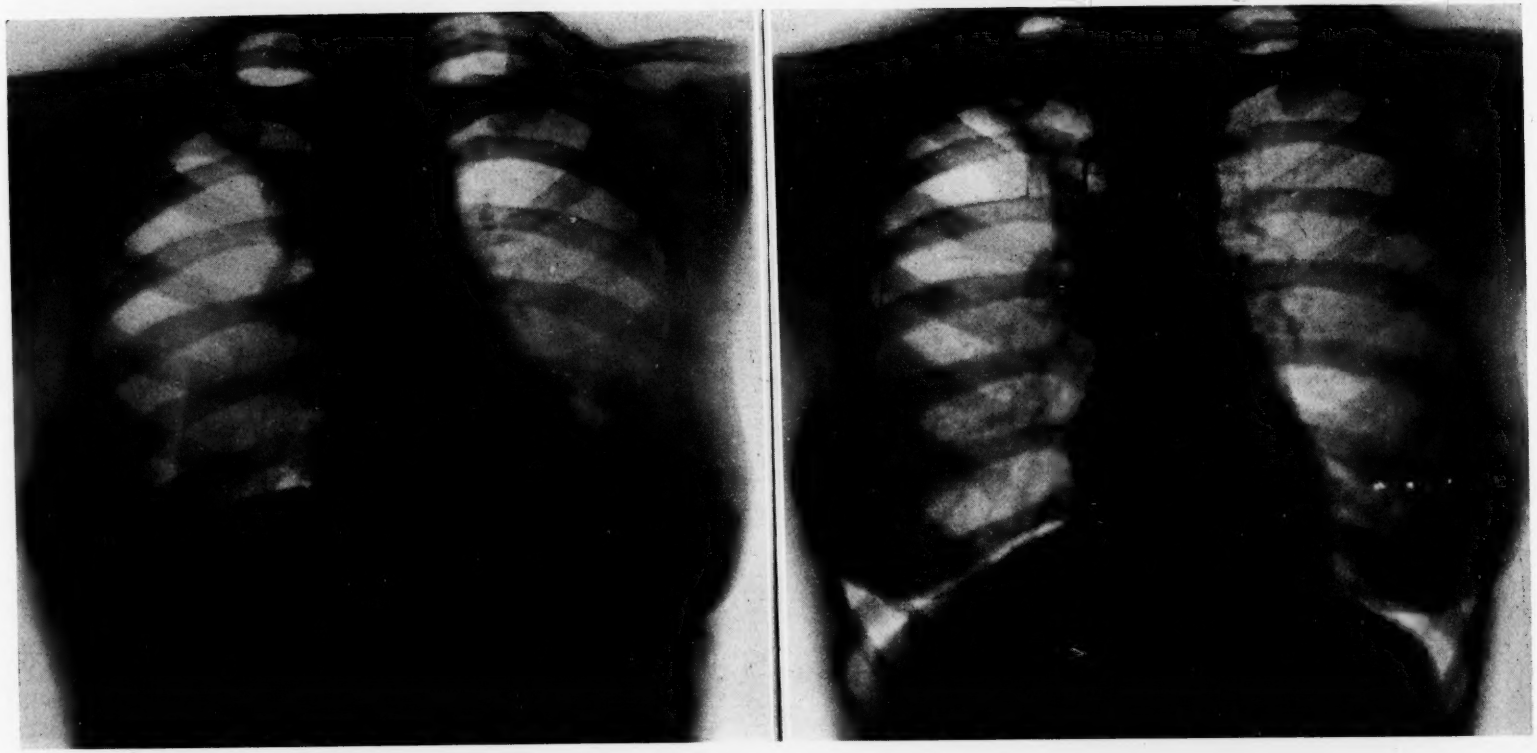

FIG. 3

FIG. 4

Taken in deep expiration and inspiration, respectively, show the collapse and subsequent cicatrization of the involved right upper lobe and the moderate compression of the lower lobes. Note how the latter are able to expand to nearly their full capacity during deep inspiration. The plates were taken one year after the induction of the pneumothorax.

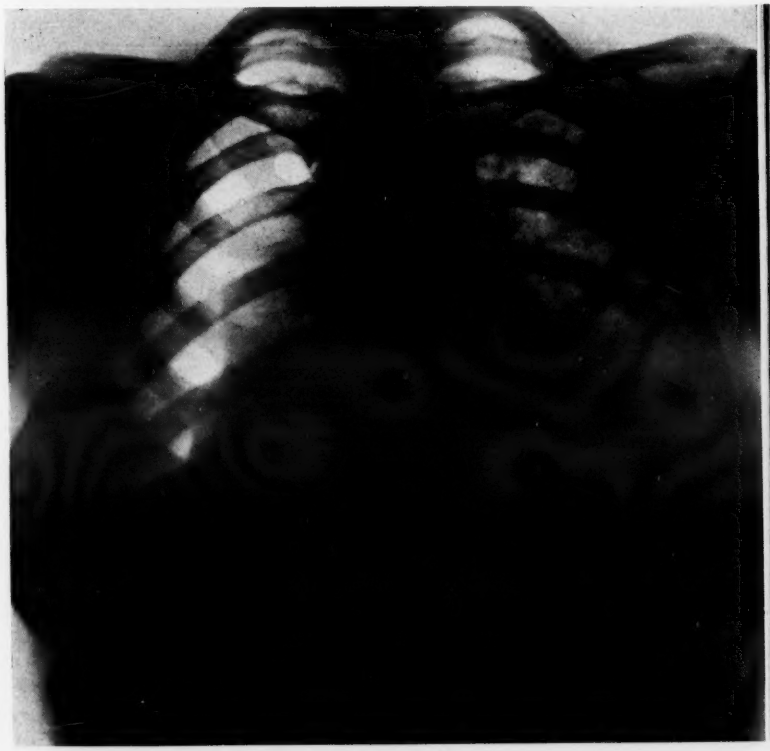

FIG. 5

FIG. 6

Taken in expiration and inspiration, respectively, show the collapse of the involved portions of the right lower and upper lobes and the moderate compression of the middle lobe. Note that the uninvolved portions of the lung, particularly the middle lobe, are able to expand to nearly full capacity during deep inspiration. The plates were taken three months after the induction of the pneumothorax.

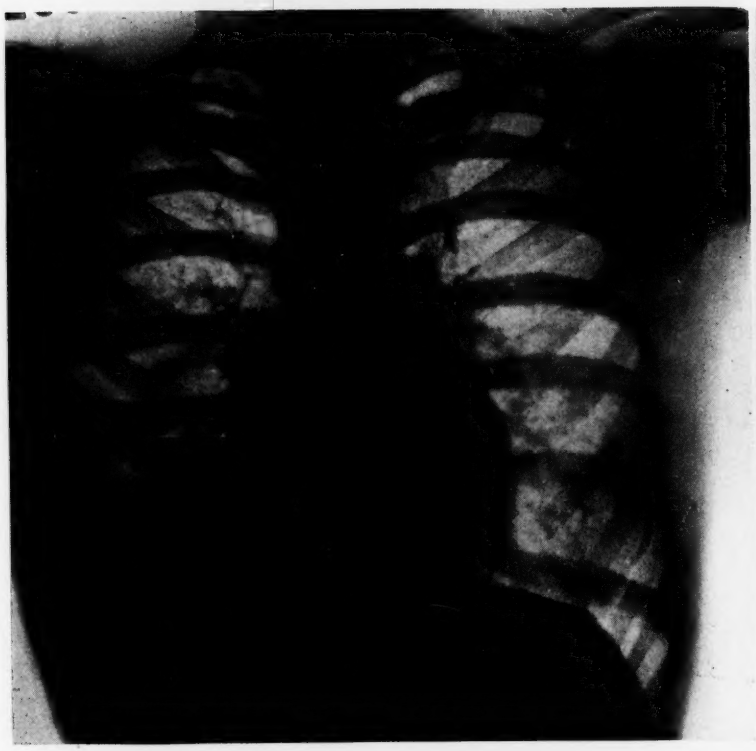

FIG. 7

Note the marked involvement of the mesial half of the lower lobe with a large cavity. The plate was taken on admission before pneumothorax treatment was instituted.

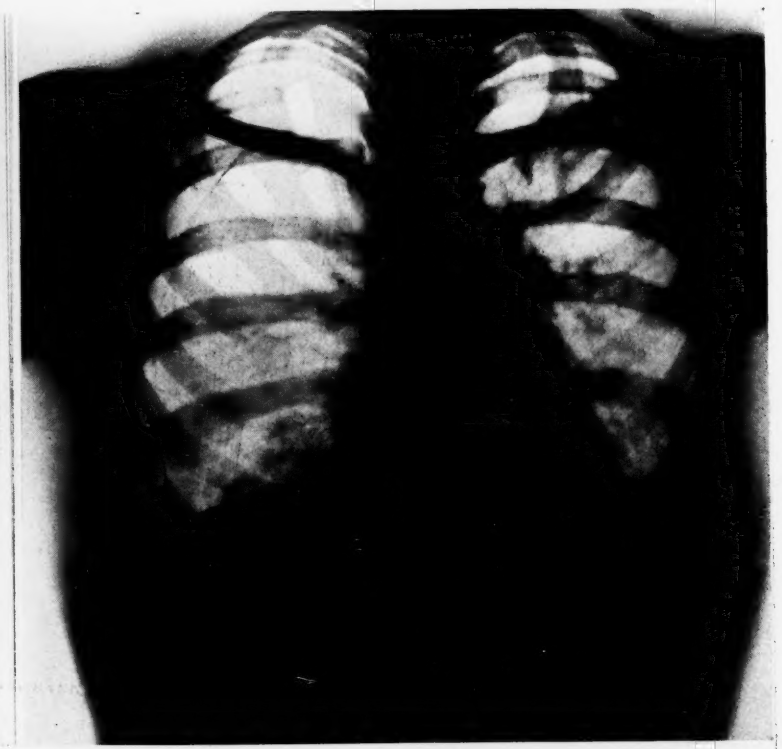

FIG. 8

A plate of the same patient as of figure 7, seven months after the induction of pneumothorax. Note the collapse of the involved portion of the lung while its uninvolved parts are permitted to function to nearly their full capacity.

FIG. 9

Note the localized pneumothorax about the involved but cicatrized upper lobe and the nearly complete reëxpansion of the lower two lobes. The plate was taken one and a half years after the induction of pneumothorax.

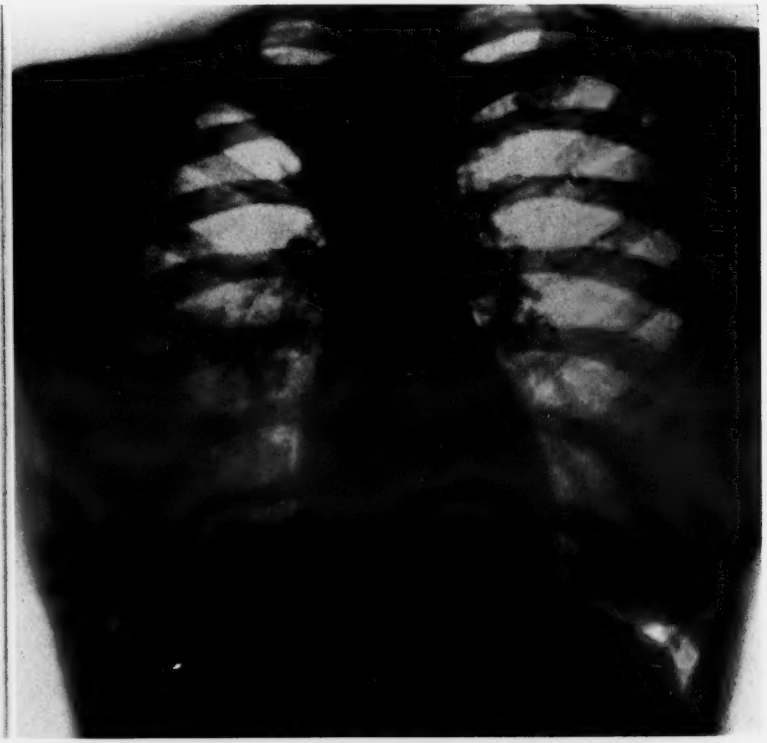

FIG. 10

A plate of the same patient as of figure 9, taken six months later. Note the tendency of the middle lobe to undergo compensatory emphysema in an effort to fill up the pleural space as the air of the localized pneumothorax about the cicatrized upper lobe is allowed to absorb at the termination of treatment. Also note the absence of mediastinal displacement.

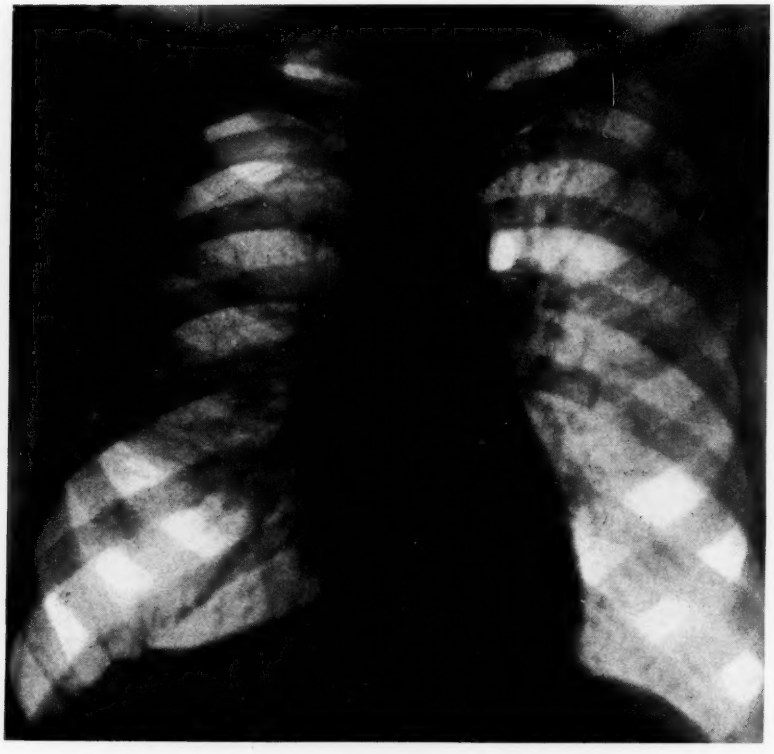

FIG. 11

A plate taken four years after induction of right pneumothorax because of an active lesion in the lower and inner portion of the upper lobe. Note localized pneumothorax about fibrosed upper lobe, excellent reëxpansion of lower lobes and absence of mediastinal displacement.

LOWER LOBE PULMONARY TUBERCULOSIS[1]

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Generalization in medicine is not infrequently illogical and at times even dangerous. Particularly is this true in the field of diagnosis. The current misconception concerning lower lobe signs in tuberculosis is a notable example of this fallacious tendency in medicine. Too commonly tuberculous involvement in the lower lobes is overlooked or misinterpreted in the absence of suggestive apical signs or, on the other hand, in the presence of equivocal signs at the apices. The comparative rarity of primary basal tuberculosis unquestionably contributes to this error: Kidd (1) states figures of other investigators at from 1 to 500 to 1 to 80, while he himself found but 2 instances in 412 necropsies of tuberculous individuals. Landis (2) categorically states that tuberculosis is never a basal condition and, futhermore, that limitation of physical signs to the bases of the lungs naturally excludes this etiology. In another text (3) Landis thus modifies this sweeping generalization: "Involvement of the lower lobe may occur very early in the disease and, although following the apical infection, may give more definite physical signs."

The diagnostic pitfall implied in this quotation led to a perusal of the early masters of physical diagnosis. It immediately became apparent that the tendency to minimize the signs in the basal lobes had had an early and illustrious advocate. For William Stokes (4) disposed of the question in the following manner:

> Now if it be asked, what gives these signs of bronchitis their value as diagnostics of incipient tubercle, the answer is, that it is not by their mere characters (for these do not differ from ordinary bronchitis), but it is from their situation, localization, and combination with comparative dullness of sound that they derive this value. The same phenomena scattered over or even existing intensely throughout the lung, but being equable and unaccompanied by dullness, would not only have no value in the diagnosis of phthisis, but would render the existence of tubercle improbable.

[1] From the Department of Clinical Medicine, University of Wisconsin.

Simple bronchitis is seldom circumscribed, while that of the consumptive is commonly so; the latter begins in the upper portion of the lung, remains obstinately fixed in the air tubes, gradually spreads downwards, and while in its first stages in the lower lobe, is combined with tuberculous ulceration in the upper; it may be intense in the upper lobe while the lower is altogether free, or engage the whole of one lung while the other is scarcely affected. These are not the characters of ordinary bronchitis.

In the main this disposition of the significance of physical signs in the basal lobes written eighty-five years ago is subscribed to by most clinicians of the present day. Stokes's treatment of the subject was in turn greatly influenced by the teachings of Laennec (5), whose classical studies on the development of the tubercle together with the attendant physical signs have come down practically unchanged through a century of growing interest and ever increasing scrutiny. Laennec wrote:

I would therefore beg to call the attention of the practitioner, to the successive development of tubercles in the different parts of the lungs. They begin to show themselves in the first place, almost always in the top of the upper lobes, more particularly in the right; and it is in these points, especially in that last mentioned, that we most commonly meet with the tuberculous excavations of vast size. It is by no means uncommon to meet with cavities of this kind, in the situation just named, when the rest of the lungs are quite sound, and do not contain a single tubercle; but in this class of cases, the patient, during life, has frequently exhibited no signs of phthisis, or only very equivocal ones, and has died of some other disease. It is much more common, however, to find one single excavation, and several crude tubercles, in a pretty advanced state, in the summit of the lungs; and the remainder of these organs, although still crepitous, and in other respects sound, crowded with innumerable tubercles, of the miliary kind, extremely small, semi-transparent, and hardly any of them with the yellow speck in the centre. It is evident that these miliary tubercles are productions of a much later date than those which had given rise to the excavations. As well from the result of my dissections, as from observations of the sick, I am well assured that this secondary crop of tubercles appears about the time when the first set begin to be softened. Very commonly we observe in the same lung evident marks of two or three successive eruptions of tubercles. Almost always, in these cases, we find that the most ancient of those which occupy the summit of the lung, have already reached the stage of excavation; that the second crop, situated around and rather below these, has already become yellow, or at least the greater part of them, but are still of no great size; that the third eruption composed of crude miliary tubercles, with some yellow points in their centre, is situated still lower;

and, finally, that the basis and inferior edge of the lung exhibit the most recent formation of all, consisting of miliary tubercles quite transparent. Exceptions to the order of development just described are by no means common. It is extremely rare for excavations to be first developed in the middle or base of the lungs; it is less unusual to find the left lung more affected than the right; it is excessively rare to find the first eruption so numerous as to prove fatal.

To this description of the pathogenesis of pulmonary tuberculosis practically the only addition in the intervening years has been Fowler's more exact localization (6) with reference to surface anatomy of the early and progressing tuberculous foci. Both Laennec and Fowler designated the apex of the lower lobe as a point second in vulnerability to the summit of the lung itself. In explanation of the predilection of the tuberculous infection for the apices of the lungs, several theories have been advanced. The propinquity of the cervical lymph nodes to the apices of the lungs and the frequency of tuberculous involvement in the former have led uncritical observers to suspect a relationship between these factors and the early primary lesions in the apices. The absence of an anatomic continuity of structures and, most important, the lack of direct lymphatic or vascular communications between the lymph nodes of the neck and the pulmonary structures in the apices refute these contentions. Such factors as ossification of the first costal cartilages may be disregarded in most cases because of the relative infrequency of its occurrence. The presence of this fixation at the thoracic operculum unquestionably bespeaks a limitation of movement of the lung beneath. But who shall term it cause rather than effect? Nature splints other inflamed parts and it may be that ossification of the costal cartilages is simply an expression of this widespread protective mechanism. Whether blood and lymph flow parallel the expansibility of the respective parts of the lung is an unsettled question, but on the premise of such a circumstance the common primary distribution of the tuberculous infection to the apices is explained. Granting the probable truth of this inferential evidence, it is not inconceivable that, exposed to the same dosage of the infection by the lymphatic or blood streams, an alteration in the expansibility of the various portions of the lung might determine a distribution of infection at variance with the rule. Against this proposed exception, no histological differences in the respective portions of the lung and their blood and lymph supply have been advanced.

Without further attempt to justify this position, the following group of four cases of pulmonary tuberculosis admitted to the University Infirmary in a period of six months is produced in order to emphasize the caution sounded by Rosenblatt (7) and others:

Case 1: S.H.: Showed slight lag in respiratory excursion at right base with increase in tactile fremitus and impairment immediately below and mesial to the angle of the right scapula. Breath sounds were somewhat harsh (expiration prolonged) over area of impairment. Sonorous and sibilant râles throughout both sides. Many fine moist râles, constant and in showers, immediately below angle of right scapula. Tubercle bacilli found in the sputum.

Stereoscopic roentgenograms show trachea slightly to the left of the midline. Heart apparently enlarged to the left. Both apices show definite but scanty parenchymal involvement, with lymphatic trunks connecting peripheral foci to hila down to second interspaces anteriorly and laterally. Upper portion of right lower lobe diffusely clouded through marked parenchymal changes. Opposite seventh and eighth ribs posteriorly is a densely walled cavity with a single trabecula partially subdividing it. Hilum lymph nodes bilaterally enlarged. (Figure 1.)

Case 2: N.H.: Definite restriction of respiratory excursion at right base. Tactile fremitus increased in area immediately mesial to and below angle of right scapula. High pitched note over entire right base. Prolonged expiration with amphoric quality mesial to angle of right scapula; bronchovesicular breath sounds elsewhere at right base. Many large bubbling râles and a few fine moist râles at right base posteriorly. Vocal resonance increased at right base. Tubercle bacilli found in sputum.

Stereoscopic roentgenograms show trachea slightly to right of midline. Heart apparently of normal size and contour. Slight clouding of left apex with a definite vertebral trunk cone connecting peripheral cloud to hilum. No definite parenchymal lesion here. A well walled cavity presents just off the right hilum from seventh to ninth ribs, posteriorly, below and lateral to which point is zone of marked parenchymal change to right base. Hilum lymph nodes are slightly increased. (Figure 2.) *Note:* Detail in the apices has been lost in reproduction. Button is extrathoracic and not a foreign body in a bronchus.

Case 3: G.S.: Reduced respiratory excursion at right base. Increased tactile fremitus between eighth and tenth ribs at right base mesial to scapular line. Tenderness on pressure in right costovertebral angle. Marked impairment in right infrascapular space. Reduction in breath sounds over area of impairment. Great showers of fine crackling and medium moist râles over this area persisting after cough. Tubercle bacilli found repeatedly after seven successive negative sputa.

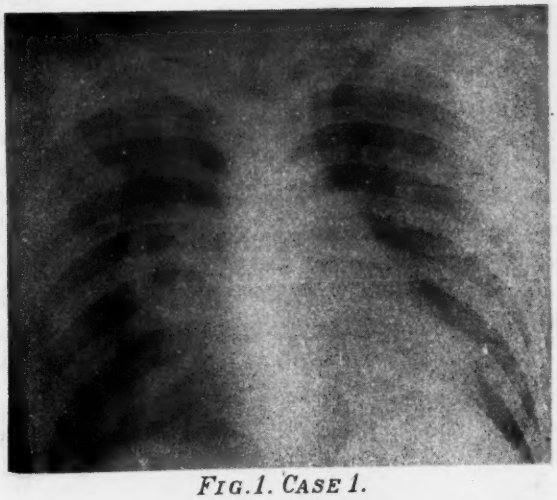

Fig. 1. Case 1.

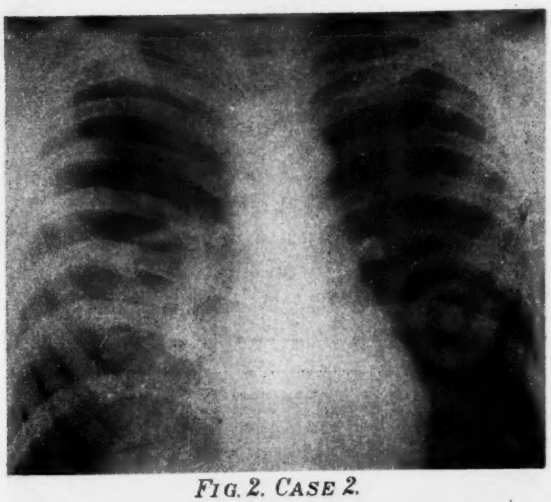

Fig. 2. Case 2.

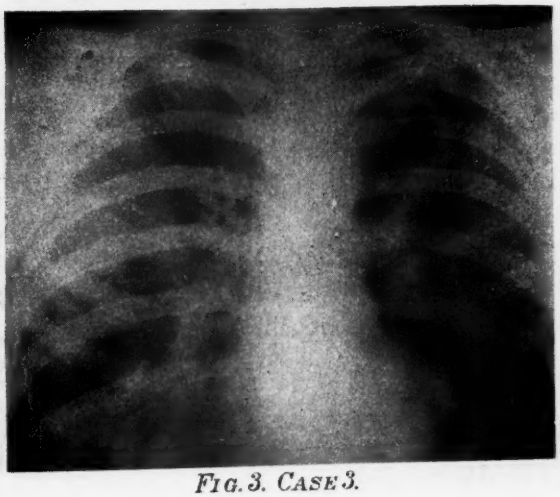

Fig. 3. Case 3.

Stereoroentgenograms show trachea slightly to right of midline. Heart apparently normal in size and contour. Diffuse but slight fogging of both apices without parenchymal changes. Vertebral and first interspace trunks bilaterally increased but do not extend to extreme periphery. Marked peribronchial involvement of a continued type about lower lobe trunks, particularly right. An area of parenchymal involvement presents posteriorly from eighth to tenth ribs on right, with soft "cotton ball" appearance. Soft changes noted in the hilum lymph nodes to either side. The diaphragms are rounded and regular. (Figure 3.)

Case 4: E.D.: Tactile fremitus increased below left scapula. Impairment immediately below and mesial to angle of left scapula. Bronchovesicular breath sounds, bronchophony and showers of medium and large moist râles heard over area of impairment. Tubercle bacilli found in sputum.

Stereoroentgenogram shows trachea in midline. Heart rather below normal size but not of a typical drop type. Diffuse, rather marked peribronchial thickening, particularly in lower lobe trunks. Right lower trunk shows unusual degree of organization, laterally, below secondary subdivision. Many small lymphoid deposits are noted at smaller branchings of bronchi in right lower lobe. Left lower lobe shows an almost congestive type of fogging, except in certain areas immediately off the hilum where characteristic "cotton ball" density pertains. Some enlargement of tracheobronchial lymph nodes with some calcific deposits on left. Domes of diaphragm rounded and regular.

DISCUSSION

Four cases of pulmonary tuberculosis are presented in which the significant physical signs were in the basal lobes. In none of these cases were there signs of pathological changes in the apices on physical examination. Case 1, on roentgenological study, showed slight, but definite tuberculous involvement of the apices. On the other hand, only equivocal evidence of tuberculous apical infiltration could be gathered from the roentgenograms in cases 2 and 3, and in case 4 the apices were absolutely clear. Contrary to the order of severity of involvement as outlined by Laennec, cases 1 and 2 showed cavities in the lower lobes. A valid objection might be raised to the inclusion of these two cases in this consideration, were it not for the demonstration of tubercle bacilli in the sputum and the subsequent rapid deterioration of both with grave hemoptyses.

CONCLUSIONS

Waiving the problematical explanation of a primary tuberculous infection of the lungs at sites other than the apices, certain deductions seem justifiable, not only from this limited study but also from the experience of the average practitioner.

1. Evident signs of pulmonary tuberculosis may be overwhelmingly or entirely basal.

2. Opposing the rule, lower lobe involvement may be much more advanced than the upper lobe focus.

3. Persistence in inflexible rules of diagnosis may lead to serious errors in diagnosis and prognosis.

4. Lastly, the arguments for a regular order of tuberculous involvement in the lung are based on the observations of many thousands of cases. The exceptions related, probably, prove the rule, and yet academic discussions of the improbability of tuberculosis in the presence of demonstrable signs in the bases alone are futile in the face of a characteristic clinical picture. The greatest care must, therefore, be exercised to eliminate a tuberculous etiology in basal as well as apical lesions.

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THE PRIMARY COMPLEX IN HUMAN TUBERCULOSIS AND ITS SIGNIFICANCE[1]

ANTON GHON[2]

Prague

The conception of the primary complex in tuberculosis originated with Ranke, who designated by the term that "definitely circumscribed" picture consisting of the primary focus of infection and the changes in the regional lymph nodes. With this term Ranke has aptly characterized the unit made up of the tuberculous changes of the primary infection and its regional lymphatic system, a unity well known to-day, not only to the pathological anatomist but to the clinician as well. Ranke's concept of the primary complex is based on the teaching of the primary lung focus, found first and more exactly studied in the tuberculosis of infants and children, forming the primary active tuberculous infection in the region of the portal of entry of the tuberculous virus in the lung, and leading to corresponding changes in the regional lymphatic system.

The basis for the teaching of the primary tuberculous focus lies in the law of Parrot, according to which every lung affection in the "virgin" body of the child shows itself likewise in the regional lymph nodes. It was Küss, chiefly, who made a detailed study of the law of Parrot, and established thereby the relationship between the site of the primary lung focus and the lymph node changes. The findings of Küss have been confirmed and extended by different authors, so that the teaching concerning the primary lung focus in tuberculosis may be looked upon to-day as one with a sound anatomic basis, standing in contradiction with no known facts in our knowledge of tuberculosis. On the basis of a large amount of anatomical material, I was able, in association with Roman, to demonstrate the fact that those changes in the lymph nodes receiving lymph from the lungs, which were the sequel of the activity of a primary lung focus, follow in their topography certain laws which correspond fully with the laws holding good for lymph drainage under normal conditions, known as the conduction and ramification law (of Bartel).

[1] Translated by Esmond R. Long, University of Chicago.

[2] Director of the Pathological Laboratory of the German University of Prague.

The conception of the primary complex of Ranke is accordingly not identical with the conception of the *primary focus* or *primary infection*, for in the primary infection we have to deal only with the changes at the point of entry of the tuberculous virus, changes around the active, local, anatomic product at the point of entry of the etiological agent of tuberculosis in the body, while the primary complex includes, in addition, the changes effected by the virus in the regional lymph nodes. The primary complex consists then of two components, of which the changes in the lymph nodes are genetically a lymphogenic metastasis of the primary infection. There can be no doubt that, according to this conception, the primary infection in point of time does precede the changes in the regional lymphatic system, that is, the lymphogenic metastatic lymphadenitis, even though the difference in the time of development is probably not a great one, since according to all that we can see and assume at the autopsy table the changes in the regional lymph nodes, in general, follow the primary infection rapidly. They constitute the first recognizable effect of the *propagation* of the virus of the tuberculosis from the primary focus, a propagation which *therefore is at first lymphogenic.*

Both components of the primary complex are variable quantities, a fact which is often emphasized in the changes in the regional lymph nodes, since the extension of the changes in the lymph nodes lying in the line of lymphatic drainage may either advance to a varying distance, or on the other hand may come to rest in one of the intermediate zones of the line of drainage. Thus, for example, the primary complex in pulmonary tuberculosis in one case, besides the lung focus, may include only changes in some regional intrapulmonary lymph nodes; in another case there are changes also in the corresponding bronchopulmonary lymph nodes in the anterior or posterior plane of the lung hilum; again, in another case changes occur, in addition, in the upper or lower tracheobronchial lymph nodes of the same side; in a fourth case, over and above these, the tracheobronchial nodes of the other side are changed; and finally changes are found in the lymph nodes lying in the line of drainage all the way up to the venous angle of one or both sides: and all this without any other focus than the primary lung nodule coming into consideration as a primary source.

Under such conditions it appears well warranted to raise the question where the limits of the primary complex can be drawn; in other words, to what point in the line of lymphatic drainage does the part of the

primary complex which comprises the changes in the lymph nodes extend? As far as I know, up to the present time, this question has not been aired by anyone else. Its answer seems to me not simple, and could depend upon various points of view. From the anatomic standpoint such a line of limitation would be possible if agreement could be reached to establish, as the boundary, a group of lymph nodes which still possessed direct lymphatic connection with the region in which the primary infection had its seat. In the pulmonary system this limit would in general be the bronchopulmonary lymph nodes and the nodes lying in the ligamentum pulmonale. Here, basically, the facts known from normal anatomy must be the determining factor. From the anatomic standpoint one could focus one's attention also upon the morphological behavior of the lymph nodes, with consideration of the characteristic given by Ranke; still the objection could be made that the duration of the process in that case played too great a rôle and did not always permit the necessary sharpness of boundary.

That the anatomic picture of the primary complex is the more definite the more locally the primary complex is limited, and the less other tuberculous changes accompany it, seems clear. Yet the complex can usually still be found when extensive secondary tuberculous changes have appeared in the lung and other organs.

Since, according to all experience, the tuberculosis which develops in human beings after birth is in the majority of instances an aerogenic infection, the *pulmonary complex* is without doubt the *commonest.* The constant and regular behavior seen in the child is found also in the adult, where, in the sense of the primary complex, we find changes either as the remains of a *primary childhood infection* or, more rarely, as a *primary late infection.* These are to be differentiated only in external form, in that the remains of the primary childhood infection consist of changes of a latent or anatomically, and sometimes biologically, healed process, while the primary late infection shows the picture of an active tuberculosis. There exist between the two named forms in adults, accordingly, differences involving the time factor, but no vital ones.

Much rarer in man are *primary extrapulmonary* tuberculous infections, in which not the lungs but some other organ of the body has furnished the primary portal of entry. Primary intestinal tuberculosis is of chief importance here, more rarely a primary oral or nasal tuberculosis, or one of the skin. Wherever the primary infection may occur, the primary complex, even when extrapulmonary, follows the same laws and principles as

the pulmonary complex, and shows also the same anatomic changes. This fact must be firmly adhered to, since to the pathologist and clinician alike it gives valuable points for determining the portal of entry in individual cases. Yet it is not to be said that the consideration of this fact will guarantee the establishment of the portal of entry in all cases. There will always be cases where a decision is difficult or impossible, without the teaching concerning the primary focus and the primary complex experiencing thereby any loss of value.

Not less significant than the diagnostic is the *immunobiological* importance of the primary complex, since it initiates tuberculous infection and constitutes the determining factor for the immunobiological relations of the body. It will act the more favorably in this respect the longer it remains as a localized process, the more slowly it extends in the regional lymphatic system, and the later it gives rise to generalization. This significance of the primary complex finds even to-day still too little appreciation. It is, however, without doubt a decisive factor for the course of tuberculosis in special cases, because, the longer the infection remains confined to the primary complex, the more readily may we increase the specific resistance to the extension of the disease, following the ideas of Petruschky. The primary complex may indeed heal *completely* as such, thereby showing the highest conceivable degree of resistance to the disease. In a larger number of cases, however, the healing of the primary complex remains no complete one. Rather, it is incomplete in that it goes over from its active stage only into one of latency. In this stage it may remain for a varying period of time, sooner or later to give rise to an endogenous reinfection, which usually has its source in the lymphoglandular components of the primary complex and can propagate in the regional lymphatic system until it breaks into the blood stream. I have designated this reinfection *endogenous lymphoglandular reinfection*. In the case of primary pulmonary infection it is no rare form of reinfection. It is significant in this case in that it can lead to hematogenic lung infection and will for the most part be unrecognized clinically, since the reinfection occurs in a field which is difficultly accessible to all clinical methods, easily recognizable as it is anatomically.

THE WILDBOLZ AUTO-URINE TEST FOR ACTIVITY IN TUBERCULOSIS

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Since Wildbolz (1) in 1919 described his auto-urine test for activity in tuberculosis, a considerable number of articles, most of which include statistical data, have appeared. Of the papers which have come to the writer's attention, only two, those by Gibson and Carroll (2), are in English. Because of this paucity of English reports and because of a lack of unanimity of opinion among workers as to the value of the test, it has seemed worth while to the writer to present his series of tests with such conclusions as he has reached.

It will be remembered that Wildbolz assumes as the basis for his test three things: (1) that there are present split products of tubercle bacilli in the urine of those harboring an active tuberculous focus in the body; (2) that these products are responsible for a specific reaction of infiltration and redness when the urine is injected intracutaneously into a person allergic to tuberculin; and (3) that, when the disease becomes inactive, decomposition products of the bacilli cease being excreted in the urine and the auto-urine test in consequence becomes negative.

Without restating Wildbolz's technique (consult Wildbolz's article (1) or abstracts in English (3 and 4)), and without detailing the findings of the various workers who have published reports, it may be noted that, in general, those working in Switzerland have corroborated Wildbolz's findings. For example, Imhof (5) of Wildbolz's clinic, considered the reaction specific, although he noted a nonspecific result in ten per cent, due, as he thought, to urinary salts. To avoid these he used auto-serum instead and claimed that while the reaction was weaker than that with urine, the serum was less irritating, and hence preferable. Lanz (6) of Leysin, in his first communication, reporting 300 tests, considered the method of value but felt the necessity of removing the urinary salts. Later (7), he gave in detail an elaborate method of dialysis and subsequent reconcentration to isotonicity which he claimed removed the salts satisfactorily and still preserved the specific substance in the

material to be injected. So far as the writer is aware this work has not been confirmed. Schippers and DeVries-Robles (8) did not think this method possessed any advantage over the original technique. A majority of those reporting agree essentially with Wildbolz as to the specificity of the test, but are not agreed as to its practical value, saying that it is tedious and beset with technical difficulties. A substantial minority disagree in principle, denying that Wildbolz has proved the test to be specific and feeling that the positive reactions may be due to one or more of a number of variable, nonspecific factors, particularly to urinary salts. Orlianski (9) claimed that these salts are more concentrated in the morning urine of tuberculous than of nontuberculous persons, and that these salts, together with nonspecific albumoses which are excreted in febrile tuberculous cases, account for positive reactions.

Wildbolz (1), of course, maintained that the specific nature of his urine reaction was demonstrated by the fact that frequently, after subsidence of the reaction, if an injection of 1 to 1000 tuberculin is made in the neighborhood, the apparently extinct urine reaction again flares up, both infiltration and redness becoming distinct. While references to this occur in the literature, the writer has not seen a report of actual confirmation of this phenomenon. Still others have corroborated Wildbolz's findings to some extent and have given a qualified endorsement of the test. Offenbacher (10) and Alexander (11), reporting an aggregate of 111 cases, concluded that the auto-urine test, when positive, constitutes fairly reliable evidence of active tuberculosis. Stiefel (12), on the other hand, after testing 400 cases, confirmed the value of a negative response as excluding active tuberculosis but thought the positive reactions inconclusive.

Obviously the questions about which the controversy has centered are: 1. Is the reaction a specific one? 2. If so, is the test suitable for practical, clinical use?

TECHNIQUE AND CASE DATA

The present series of tests, while small, was carefully done, the preparation of the urine being supervised and the injections and readings being made by the same person throughout. The technique adhered to that of Wildbolz (1) in 49 cases, while in the other 32 v. Bergen's slight modification (13) was introduced; that is, instead of concentrating the urine uniformly to one-tenth its original volume, it was concentrated according to an empirical standard depending on its specific gravity. As

the results were essentially the same with the two technical procedures, separate tables are not given. Care was particularly exercised that the urine during evaporation should not be heated above 55°C., in spite of the fact that certain workers (v. Bergen (13), Miche (14)) have considered the specific substance thermostable. The concentrated product was uniformly kept on ice over night before filtering and injecting. Very minute amounts of the clear filtrate, usually 0.05 cc., were used for injection, the same amounts of O. T., 1 to 1000 and 1 to 10,000, being used to test for allergy. The attempt was consistently made, using always a 28 gauge needle, to inject to a constant depth, although this was, manifestly, difficult of accomplishment. Readings were made after twenty-four and forty-eight hours, and only the presence of a palpable nodule, persisting at least forty-eight hours, was considered a positive reaction. No attempt was made to correlate the degree of infiltration with the degree of activity of the tuberculous process as this did not seem feasible.

Excluding those cases in which, during observation, the presence or absence of active tuberculosis was in doubt, and those anergic to tuberculin in which confirmatory tests on allergic patients were not made, there remain 81 cases in the series. These consisted, with three exceptions, of Chinese, were of both sexes and ranged in ages from four to fifty-four years. The results of the test are given in table 1.

The cases considered to be actively tuberculous numbered 33 and were distributed regionally as follows: lungs 16, bones and joints 11, lymph nodes 3, peritoneum 1, skin 1, adrenals 1. In the lung cases symptoms and signs were checked with laboratory findings, including X-ray examinations. In all the others, except the adrenal case, histological evidence was relied upon. The last-named case, while clinically one of Addison's disease, did not come to autopsy; hence no absolute proof as to its nature was obtained. Of the 33 cases, 29 agree with Wildbolz's results and 4 disagree. The case of Addison's disease was not among these four. In the two cases in which the tuberculin test was positive but the auto-urine test was negative kidney function was good. The cases considered to be nontuberculous numbered 48. These were carefully questioned concerning symptoms of tuberculosis, past and present, and, in addition to receiving routine physical examinations, were examined particularly with tuberculosis in mind. X-ray examinations and repeated subcutaneous injections of old tuberculin were resorted to freely. Of these 48, 31 responded negatively to the auto-urine test and 17 positively. A majority of the diagnoses in the latter group are found in the former

group also. It will suffice here to enumerate the primary diagnoses of the 17 cases reacting positively. They are two of osteomyelitis and one each of schistosomiasis, cellulitis, organic heart disease, hemolytic streptococcic arthritis, gout, cerebral syphilis, chronic opium poisoning, hypothyroidism, cirrhosis of the liver, pernicious anemia (atypical), lobar pneumonia, chronic bursitis, carcinoma of the esophagus, chronic dysentery (bacillary), and no disease. All but three were afebrile and the specific gravity of their morning specimens of urine did not average higher than that of those reacting negatively.

TABLE 1

Showing the skin reaction to tuberculin and auto-urine in 33 tuberculous and 48 nontuberculous cases

	SKIN REACTIONS	AGREEING WITH WILDBOLZ	DISAGREEING WITH WILDBOLZ
33 actively tuberculous cases	O.T.*+, A.U.+	27	
	O.T.−, A.U.−, but A.U.+ in allergic patient	2	
	O.T.−, A.U.+		2
	O.T.+, A.U.−		2
48 nontuberculous cases	O.T.+, A.U.+		16
	O.T.−, A.U.−, and A.U.−in allergic patient	4	
	O.T.−, A.U.+		1
	O.T.+, A.U.−	27	
Total—81 cases		60 or 74 per cent	21 or 26 per cent

* O.T. signifies old tuberculin reaction.
A.U. signifies auto-urine reaction.

DISCUSSION

The questions of interest still are: (1) Is the test specific for active tuberculosis? and (2) if so, can it be employed practically in clinical work? The second question is more easily answered than the first. From personal experience the writer would say that the test fails in at least three particulars to fulfil the conditions necessary for its acceptance by physicians, whether in private or hospital practice. It requires a considerable amount of apparatus, it is time-consuming and the chances for technical error are great. For these reasons it would seem that the test, according to the Wildbolz technique, is not suited for general use.

As to the first and most interesting question, it is premature to dogmatize. It would harmonize with current conceptions of immunity to

assume with Wildbolz that in active disease the disintegrated products of tubercle bacilli find their way into the blood and subsequently, in part, are excreted by the kidneys; further, that these products should be antigenic if reinjected parenterally. There are, however, two or three points that should be borne in mind when considering activity and allergy in tuberculosis.

One wonders, for example, how constantly the split products of the bacilli are to be found in the blood and urine during active disease. The best guide to activity in tuberculosis is constitutional symptoms. These, however, may be due, according to Krause (15) to intoxicating substances released by the disintegration not only of bacilli but also, and to a greater degree, of tubercle cell products. Moreover, in both instances these act nonspecifically. Hence it is possible that the urine in actively tuberculous cases contains predominantly certain of the disintegrated cellular elements of the tissues, with only an insignificant amount of the bacillary elements. Indeed, at times the former might be present to the exclusion of the latter. If, under such circumstances, skin allergy to auto-urine be present it would seem necessary to attribute to nonspecific degradation products of tissue cells alone (or of cells and bacilli) the property of being able to evoke specific skin reactions.

Assuming, for the moment, that bacillary elements are constantly present in the urine during active tuberculosis, the question arises as to their state of disintegration. Have they become so thoroughly split up that they are no longer capable of producing skin reactions? v. Bergen (13) and others showed the urinary substance under consideration to be biuret-free, and considered it to be of a polypeptid nature. It has apparently been demonstrated by Zinsser (16) that hypersensitive reactions may be elicited by intracutaneous injections of bacterial extracts from which all coagulable proteins, nucleoproteins and Bence-Jones proteins have been removed, as well as this can be done by boiling with acid, etc. But there must be a limit beyond which further simplification of the molecule of bacterial bodies or extracts will cease to act in a specific manner.

It is proper to associate allergy with immunity in tuberculosis. Allergy to tuberculoprotein always comes into being through the establishment of focal tubercle, and in this way only (Krause (17)), and immunity in susceptible laboratory animals is likewise secured. But all the known antibodies may be absent in animals which are demonstrably immune to

tuberculosis and can be present without antecedent tubercle or infection with tubercle bacilli. Hence, the unwisdom of necessarily linking up antigen-antibody reactions with activity in tuberculosis.

Aside from these theoretical considerations, one is confronted with a very practical difficulty when applying the test after the Wildbolz technique. The nonspecific irritating elements of the urine which are water-soluble, such as pigment, urea, chlorides, oxalates, sulphates and phosphates, are very much concentrated in the solution injected, and often cause reactions indistinguishable from the so called specific reactions. If Lanz's work (7), above referred to, were confirmed in large series of cases of various stages of tuberculosis, with numerous controls, the specificity of the Wildbolz test would be less open to question.

SUMMARY AND CONCLUSIONS

1. The literature concerning the Wildbolz auto-urine test is briefly reviewed.

2. A series of 81 cases, 33 actively tuberculous and 48 nontuberculous, were given the auto-urine test, of which 74 per cent agreed and 26 per cent disagreed with Wildbolz's findings.

3. Urine contains irritants which render it unfit, particularly when concentrated, for delicate tests for skin hypersensitiveness. Until these nonspecific elements are eliminated as a factor in the test, and evidence is then secured on a large scale, it is premature to call the Wildbolz test specific.

4. Certain theoretical considerations are presented which lead one to question the specificity of the test.

5. In its present form the Wildbolz test is not practically helpful, inasmuch as it would be unsafe in doubtful cases to use the test as a criterion of activity.

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THE WILDBOLZ AUTO-URINE REACTION

A REPORT OF SEVENTY-FIVE CASES[1]

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In May, 1919, Wildbolz (1) described a test for the recognition of active tuberculosis. This test, based on the work of Maragliano and Marmorek, assumes that when there is an active focus of tuberculosis anywhere in the body the products of elimination and destruction of the tubercle bacilli will circulate in the blood, and a fraction be eliminated in the excretions of the body. If this is so, it should be possible to demonstrate these bodies in the urine of a tuberculous subject by means of the allergic reaction of the skin; that is, upon injecting into his skin a minute amount of his own urine, properly prepared, an allergic reaction should be obtained similar to that resulting from the intracutaneous injection of tuberculin. The urine thus acts as a weak autogenous tuberculin.

As the products of tubercle bacilli can be eliminated only while there is an active focus in the body or shortly afterward, a positive test will be obtained only in active cases of tuberculosis, thus differing from the conditions necessary for the ordinary positive tuberculin tests. Again, in the case of Wildbolz's test, the antigen used to demonstrate skin hypersensitiveness is derived from the patient himself, instead of from tubercle bacilli grown *in vitro*. An anergic patient, or one who does not react to tuberculin, cannot give a positive result.

The test has attracted quite a little attention in Germany during the past three years, but up to the first of this year I have seen papers by only two workers in this country (Gibson and Carroll (2)).

Wildbolz was unable to demonstrate any antigen in unconcentrated urine. Believing it to contain the most antigen, he therefore concentrated morning urine to one tenth its volume, at a temperature not exceeding 65 to 70°C., and low enough to prevent the destruction of the antigen. Bergen (3), Grosjean (4) and Miche (5), however, maintained

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that the antigen is thermostable, while Katzulla (6) held that the lower temperature is necessary to prevent the formation of irritating compounds from the urinary salts. The concentrated urine is then filtered and a minute amount injected intracutaneously.

Our technique is practically that used by Gibson and Carroll, which includes some slight modifications of Wildbolz's original method. Obtained as far as possible under sterile conditions, 150 cc. of freshly voided morning urine is evaporated to one-tenth its volume in a litre side-arm distilling flask, in the neck of which a thermometer is so adjusted that its tip just touches the surface of the liquid when evaporation is complete. The distilling flask is connected to a condensation bottle. High pressure rubber tubing runs from this to an ordinary water filter pump by which the vacuum is obtained. Between the pump and condensation bottle are interposed a trap bottle, a closed mercury manometer, and a capillary tube for controlling the vacuum.

When a vacuum of 28 inches is obtained, the distilling flask is placed in a water bath and evaporation begun. If the temperature rises above 50° C. or the pressure falls to a 27 inch vacuum, the water bath is removed until the required vacuum is again established.

When concentrated to one-tenth its volume the urine is decanted into a sterile tube and placed in the icebox over night, when most of the urinary salts are precipitated. These are removed by filtration through sterile filter paper moistened with 2 per cent phenol solution. The sterility of the product is tested by transferring 0.5 cc. to broth media. (We have not obtained any growth except when the urine contained large numbers of colon bacilli or staphylococci before evaporation, although the more refined methods for the presence of bacteria were not employed.) The preparation is now ready for use.

A minute amount (0.05 cc.) is injected intracutaneously on the anterior aspect of the forearm. Two control injections, in equal quantities, of 1 to 1000 solution of O. T. and a control urine are made at the same time. The tuberculin solution is used to test the allergy of the patient. The control urine of a nontuberculous individual, who did not react to his own urine, was prepared in the same way as the test urine, and was used to estimate the degree of reaction to the latter. This was not done by Wildbolz, but was suggested by Eliasburg and Schiff (7), Boeninghaus (8) and Gibson and Carroll. It is of great importance, for no satisfactory interpretation of the degree of reaction can be made without it.

A positive reaction consists of a sharply circumscribed infiltration in the skin, 5 to 10 mm. in diameter, appreciated more by the sense of touch than by sight, and often, but not always, accompanied by slight redness. Infiltration is much more important than redness, for the latter may be absent in typical cases, or present when badly contaminated nontuberculous urine is used. The infiltration generally reaches a maximum in twenty-four to forty-eight hours, and gradually fades during the following four or five days. Occasionally the infiltration does not appear until three or four days after injection. As different patients show different degrees of reaction to the control urine, a positive reaction must show appreciably more infiltration than the control. The degree of reaction, however, is not proportional to the severity of the case.

The injection must be intracutaneous. Subcutaneous injection will fail to elicit a reaction in cases which do react intracutaneously. On the other hand, too superficial an injection may give a superficial skin necrosis, making interpretation much more difficult.

Various modifications of the technique have been suggested. Thus Lanz (9) removed the irritating urinary salts by dialysis. Alexander (10) insists on removing all albumen before concentration, to insure against a nonspecific reaction. He also tried concentrating the urine at ordinary temperatures, but found it did not give as many positive reactions as that concentrated at reduced pressure. Unconcentrated urine never gave a positive test. Grosjean and Bergen regulated the degree of concentration according to the specific gravity of the urine. Bosch (11), after concentrating the urine, precipitated the salts with 20 volumes of 90 per cent alcohol and then concentrated it again. Schmid (12) urged repeated tests if the first was negative, for the urine may be temporarily antigen-free.

Imhof (13) used a concentrated blood serum instead of urine, and reported similar results. Nasso (14) applied the test in cases of meningitis, using spinal fluid concentrated to one-tenth its volume, and believed a positive reaction pathognomonic of tuberculous meningitis. Several workers use a 1 to 10,000 as well as a 1 to 1000 O. T., and many omit the control urine.

What experimental evidence is there for the specificity of the test? Alexander injected two guinea pigs with the concentrated urine of a tuberculous patient who gave a strongly positive test. The pigs gave no reaction. They were then inoculated with tuberculous material and

fourteen days later showed a definite infiltration at the site of the original inoculation. Schmid obtained a positive reaction with urine from a tuberculous patient injected into another patient who reacted strongly to 1 to 10,000 tuberculin, but who did not react to his own urine. Cepulic (15) observed that 0.2 cc. of the concentrated urine of a tuberculous patient injected into another sick tuberculous patient produced the general and local reactions of tuberculin, while with the urine of a clinically tuberculosis-free patient no such reaction was obtained. Wildbolz noted a fading urine reaction flare up again when 1 to 1000 O. T. was injected near it. This has been confirmed by Gibson and Carroll.

Weiss (16), however, failed to obtain the same results by the injection of fractional precipitates of the concentrated urine as he did when treating a dilute tuberculin solution in the same way, and Trenkel (17) obtained the same results with an "artificial urine" and urine concentrated at ordinary temperature as he did by the Wildbolz technique.

The conclusions of the various observers on the reliability of the test are by no means in accord. Thus, of 26 papers, 11 are favorable, 12 unfavorable and in 3 judgment is reserved. In many cases the unfavorable results are undoubtedly due to errors in technique and lack of controls, and to the interpretation of slight and doubtful reactions. Thus, Wildbolz regards a reaction equal to that of 1 to 10,000 tuberculin as positive, while Offenbacher (18) regards it as doubtful.

The unfavorably impressed workers believe the test specific, but antigens too dilute (Lieberhardt (19), Farago and Randt (20)), or that the reaction is due entirely to the urinary salts present (Reinecke (21), Levi (22), König (23), Orlianski (24)).

On the other hand, in his original paper Wildbolz reported no positives in a series of 50 controls, and positive reactions in all of a series of 107 proved cases of tuberculosis, except two cases of bilateral renal tuberculosis, in which the kidney function was greatly impaired. Lanz also obtained positive tests in all cases of bone tuberculosis, but none in definitely nontuberculous bone disease. Gibson and Carroll, Stiefel (25) and Bosch, Alexander and Kipfer (26) have all reported favorable sesults in large series of cases, while Heimberger (27), Bressel (28), Andreasch (29) and Katzulla have had the same experience with smaller series.

Kipfer gives a summary and analysis of most of the reported work up to December, 1922. Of 1443 clinically active cases of tuberculosis, tested

by 26 different workers, 90.1 per cent gave a positive auto-urine reaction and 9.9 per cent a negative reaction. If anergic cases (40) are excluded, the percentages are 92.5 per cent and 7.5 per cent. Of 845 cases without clinical evidence of tuberculosis, 31.2 per cent gave a positive and 68.8 per cent a negative test. This includes the series of cases in which proper controls were not used.

Kipfer's own series included 200 cases. Of 109 cases of active tuberculosis, 104 or 95.5 per cent were positive, and 5 or 4.5 per cent negative (including 3 anergic cases). Of 66 nontuberculous patients, 12 or 18.2 per cent were positive, and 54 or 81.8 per cent negative; and of 25 cases of clinically healed tuberculosis, 12 or 48 per cent were positive, and 13 or 52 per cent negative. He believes that clinically healed cases which still give a positive auto-urine reaction bear further watching.

Our own series includes but 75 cases, consisting of 38 cases of proved tuberculosis, 7 doubtful, and 30 supposedly nontuberculous patients as controls. Of the 38 cases of proved tuberculosis, 29 or 76.3 per cent gave a positive reaction, 3 or 7.8 per cent a doubtfully positive reaction, and 6 or 15.8 per cent a negative reaction. Reactions classed as positive showed an infiltration at least 5 mm. greater than from control urine; the doubtfully positive, less than 5 mm. Of the 30 control cases, 23 or 76.7 per cent gave a negative test, 6 or 20 per cent a doubtful, and 1 or 3.3 per cent a positive reaction. One of the doubtful reactions was in an anergic patient, whose urine gave a doubtful positive reaction in another allergic tuberculous patient.

The series includes 31 cases of pulmonary tuberculosis with positive sputum. Of these, 27 were allergic, or gave a positive reaction to 1 to 1000 O. T. Of these 27 cases, 22 or 81.5 per cent gave a positive auto-urine test, 2 or 7.4 per cent a doubtful, and three or 11.1 per cent a negative test. One of the patients giving a negative test had, in addition to pulmonary tuberculosis, a chronic osteomyelitis of his right femur and at autopsy showed advanced amyloid disease of the kidneys. Four cases of questionable pulmonary tuberculosis were all negative.

The urine of 3 of the 4 anergic patients gave a positive reaction when injected into an allergic individual. The urine of the fourth patient was not so tested.

Three of five cases of proved bone tuberculosis were positive, while four doubtful cases, although clinically tuberculous, were all negative.

One case of tuberculous salpingitis and one of renal tuberculosis gave positive reactions.

We believe that a definitely positive reaction, properly controlled, is indicative of active tuberculosis and that a negative test is of less value unless repeated. Doubtful reactions, when the degree of infiltration to test and control urines are approximately the same, must be interpreted with extreme care and are of little or no value in diagnosis.

The test, while theoretically sound, is beset with so many sources of error in its present form that a reliance cannot be placed upon it equivalent to the clinical criteria at present in use. It may, however, be useful to corroborate the clinical diagnosis. It is worthy of further trial and an attempt should be made to devise a simpler and more reliable technique.

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A COMPARISON OF THE SODIUM HYDROXIDE AND ANTIFORMIN METHODS FOR CULTIVATING TUBERCLE BACILLI

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There are many occasions when it becomes desirable to cultivate large numbers of individual strains of tubercle bacilli with the highest attainable efficiency and the elimination of time-consuming manipulations, not to mention the exclusion of contaminators. The guinea pig inoculation method for obtaining pure cultures requires a month or more before the tuberculous tissue, free from contamination, can be obtained in form suitable for seeding inspissated serum tubes. It was shown by Dorset that inspissated egg could better be substituted for serum media. With the advent of antiformin as an antiseptic it was further realized that rapidly growing microörganisms were far more susceptible to its action than were wax-protected tubercle bacilli. Realizing the importance of this for cultivating tubercle bacilli directly from contaminated sources and thus eliminating the time of waiting for the guinea pig to develop tuberculosis, Griffith utilized this reagent and accomplished in about five to thirty minutes what previously had required weeks.

The criticism of the original Griffith method was that the antiformin acted so quickly that sufficient time was not allowed for the manipulation and washing of the material to be cultivated, so that the reagent would frequently destroy the tubercle bacilli before they could be safely seeded on the medium alive. In Griffith's contribution of 1914 (1) the method of treatment of the sputum was given as follows:

> After trial of various proportions of sputum, antiformin and diluent (physiological salt solution) the method employed for some little time has been as follows: 1 cc. of thick mucopurulent sputum was pipetted into a sterile centrifuge tube (when the sputum was mucoid and contained much saliva, 2 or 3 cc. were taken). To this, salt solution to make about 9.5 cc., and then 0.5 cc. antiformin were added; the sputum was then stirred up, and after five, ten, or fifteen minutes, according to the speed with which the sputum dissolved, the mixture was centrifugated. The deposit was washed with salt

solution, recentrifugated, and then sown upon four tubes of egg medium. This washing was later found to be unnecessary, the amount of unreduced antiformin lingering in the deposit being insufficient, apparently, to interfere with the growth of the tubercle bacilli.

Finally this was again modified so as to use the whole sputum treated with antiformin long enough to kill contaminating microörganisms as follows:

Equal parts (0.25 cc. is sufficient) of a 10 per cent dilution of antiformin and mucopurulent sputum were pipetted into a small test tube and thoroughly mixed. After the lapse of from five to thirty minutes, tubes of egg medium were sown, each with a loopful or two of the transparent brownish mucinous mixture, and were incubated in the usual way. Treatment for five minutes with 5 per cent antiformin was not always successful in destroying contaminating organisms, though these succumbed to longer exposure. . . . . The simple method should, perhaps, be restricted to sputa in which the tubercle bacilli are numerous. When tubercle bacilli can be demonstrated microscopically in small number only or not at all, the centrifugating method by which the tubercle bacilli present are concentrated into small bulk is more likely to succeed. . . . . The presence of free antiformin in material used for sowing cultures has been proved by control experiments to be not detrimental to the growth of tubercle bacilli. . . . . Tuberculous glands mixed with 10 per cent antiformin revealed a few bacilli still alive at the end of twenty-four hours.

In a paper in 1916 (2) he again states:

that of 31 sputums cultivated by the antiformin method pure cultures resulted from 26 of the samples and that there is no need to centrifugate the bacilli and wash them free from antiformin before sowing. . . . . In fact, it is inadvisable in cultivation experiments to allow an antiformin sputum mixture to become so fluid that it is possible to centrifugate the bacilli. The method is carried out as follows: Equal volumes of dilute antiformin and sputum are pipetted with a wide-bore pipette into a small test tube and thoroughly mixed. At intervals subsequently one loopful of the mixture is spread evenly over the surface of egg medium and the tubes are then incubated. By means of this simple procedure I obtained pure cultures of tubercle bacilli from 26 of the 31 new samples of sputum. In each experiment the volume of the sputum taken was about 1 cc., and this was mixed with an equal volume of a 10 per cent dilution of antiformin, thus forming a 5 per cent antiformin, sputum mixture. Egg tubes were sown with this mixture at intervals of five (sometimes ten) minutes during the first thirty minutes, the

first tube being sown five minutes, occasionally ten minutes, after the mixture was made. In all the 26 positive experiments the mixtures were free from living organisms other than tubercle bacilli in twenty minutes or less. In 12 experiments pure cultures of tubercle bacilli were obtained after the antiformin had acted for about ten minutes and in 7 experiments after exposure for only five minutes. On the other hand, tubercle bacilli themselves did not long resist the action of 5 per cent antiformin. In 13 experiments mixtures which yielded pure cultures of tubercle bacilli after ten minutes' exposure to 5 per cent antiformin were sterile after further periods of five to ten minutes. It is evident from these results that with thin sputa the margin of time available during which pure cultures of tubercle bacilli are possible from a mixture may be very brief. When the sputum was thick the tubercle bacilli escaped early destruction, and such mixtures were found capable of producing cultures twenty-four hours after the sputum and antiformin were mixed.

Finally, Griffith recommends, for routine use, 15 per cent antiformin in the sputum mixtures and the first sowing to be made in one to two minutes after mixture, according to the consistency of the sputum. Three tubes ought to be sown within the first five minutes. Thereafter the intervals may be longer (five minutes), especially if the sputum remain undissolved and mucinous. There is no need to sow tubes after twenty minutes or after the sputum has completely dissolved.

Griffith's statements impress one strongly with the high susceptibility of tubercle bacilli to antiformin and the difficulty in laying down even an approximate standard technique, so that at the outset one feels that the saving of time in utilizing antiformin is sacrificed subsequently in the necessity for having many time intervals and seeding large numbers of tubes of medium which, to say the least, is also time-consuming.

Lyall (3) utilized this method and reported good results, highly recommending the method. He admits that the method proposed by Petroff (4) constituted a step forward, but did not greatly reduce the manipulations necessary and without the use of gentian violet medium did not yield as high a percentage of noncontaminated tubes as could be desired. He failed to add the gentian violet to the medium, which was incorporated by Petroff to inhibit contaminators not destroyed by the sodium hydroxide, and gave no comparative data on the antiformin and sodium hydroxide methods. In 55 out of 56 specimens tubercle bacilli were isolated in pure culture by the Griffith direct method without centrifugating and less than 5 per cent of a large number of tubes (1080) showed any contaminations.

In 1918 Corper, Fiala and Kallen (5) routinely cultivated 526 positive sputums, using Petroff's method, and obtained varying grades of cultures on primary culture from 144 (27.3 per cent). Petroff (6) himself reported as high as 129 positives from 135 specimens, a figure probably slightly overestimated, in view of the fact that there is a distinct difference in the growth ability of different strains of human tubercle bacilli freshly isolated from the sputum and those artificially cultivated for long periods upon media prepared by adding various nutrients to a basic medium composed of agar, ammonium phosphate, salt and glycerol. Corper found that a few strains of recently isolated cultures failed to grow on all the media in spite of heavy seeding from culture tubes and 3 of 28 recently isolated cultures failed to give appreciable growth on any of the 16 media used, while 9 gave growths only on a few media, 6 of these being on whole egg and 5 on rabbit's blood (7); while Theobald Smith observed (8) that a culture may contain sufficient viable bacilli to infect a highly susceptible animal like the guinea pig and still not grow on artificial media. Even relatively young cultures on glycerol bouillon fail to grow when transferred to glycerol agar. The larger the mass of bacilli transferred, the greater the chance for successful subcultures, very fine suspensions failing to grow. That, in the Petroff method, even higher concentrations than an equal volume of 3 per cent sodium hydroxide at 37°C. for thirty minutes to one hour can be utilized with safety is indicated by the work by Corper (9) and Soparkar (10). The latter found that an equal volume of 5 per cent sodium hydroxide at 37°C. for one hour gave a good growth, equal to controls, while, after this, growth was less profuse but was still present at eighteen and twenty-four hours, and practically all contaminators were killed in ten minutes by the alkali; while Corper found that an equal volume of 6 per cent sodium hydroxide may affect the virulence of some cultures, as tested on guinea pigs, after one hour at 37°C. Soparkar prefers the sodium hydroxide to the antiformin method. Regarding other details of technique such as paraffining the cotton stoppers, capping tubes with tin-foil to prevent evaporation, and the effect of the 1 to 10,000 dilution of gentian violet in the egg medium, Goodman and Moore (11) found that when this medium was seeded fairly heavily with any one of three human strains or of two bovine strains, all accustomed to artificial cultivation on glycerol agar or Petroff's gentian violet-egg medium, growth could be expected on practically all the uncontaminated tubes within two months.

In view of the complete lack of comparative data on the antiformin and sodium hydroxide methods for the cultivation of tubercle bacilli from sputum and contaminated material, and the apparent distinct advantages of the antiformin method, as finally devised by Griffith, both as a time-saving factor in not requiring centrifugating or washing and in the ease of performance, it seemed highly desirable to study these two methods, utilizing for this purpose the same suspensions of tubercle bacilli and the same contaminated material, that is, sputum, and seeding the treated material upon the same culture media, namely, Dorset's egg medium and Petroff's gentian violet-egg medium. The problem finally divided itself into determining the effect of these reagents upon suspensions of tubercle bacilli themselves, and the effect upon the contaminators. In order to determine the effect upon the tubercle bacilli themselves, two experiments were performed. In one the action of an equal volume of different concentrations of sodium hydroxide from 1 to 6 per cent, for thirty to sixty minutes at incubator temperature, upon suspensions of human tubercle bacilli in physiological saline solution was studied; in the other the action of an equal volume of 10 and 20 per cent antiformin for 2, 5, 10, 20 and 30 minutes and 1 hour, at room temperature, upon the same tubercle bacilli was studied. The results of these two experiments are recorded in tables 1 and 2, respectively. In the sodium hydroxide experiment the bacilli were centrifugated and the sediment washed free from alkali with three changes of sterile physiological saline solution, after which the sediment was seeded on to the medium, while in the antiformin experiment the sediment was seeded upon the media directly, after centrifugating and without washing.

The results recorded in table 1 indicate that suspensions of human and bovine tubercle bacilli in physiological salt solution grow well after treatment for thirty and sixty minutes at 37°C. with an equal volume of sodium hydroxide up to 6 per cent concentration, and washing and seeding the washed and centrifugated sediment. Those recorded in table 2 indicate that suspensions of human and bovine tubercle bacilli in physiological salt solution grow well after treatment for two minutes to one hour at 20°C. with an equal volume of 10 and 20 per cent antiformin, and seeding the centrifugated sediment without washing. Growth is obtained equally well on Dorset's egg medium or Petroff's gentian violet-egg medium.

In view of the apparent harmlessness of the sodium hydroxide up to 6 per cent for thirty to sixty minutes at 37°C. and the antiformin up to

20 per cent for one hour at 20°C., added in equal volume to suspensions of tubercle bacilli, and the lack of importance as to whether Dorset's egg medium or Petroff's gentian violet-egg medium was used for obtaining

TABLE 1

Action of sodium hydroxide upon suspension of tubercle bacilli in salt solution

STRENGTH OF NaOH IN PER CENT ADDED TO AN EQUAL VOLUME OF BACILLUS SUSPENSION	TIME OF EXPOSURE TO NaOH AT INCUBATOR TEMPERATURE	TIME OF INCUBATION BEFORE READING CULTURES IN WEEKS	CULTURE TUBES WITH GROWTH OF TUBERCLE BACILLI*		
			Gluckson	Human	Bovine virulent
	minutes				
1	30	4	3	3	2
		8	3	3	3
	60	4	0		3
		8	1		3
2	30	4	0	3	3
		8	3	3	3
	60	4	0	2	3
		8	0	3	3
3	30	4	2	3	3
		8	3	3	3
	60	4	0	2	3
		8	3	3	3
4	30	4	0	2	3
		8	2	3	3
	60	4	0	2	2
		8	3	3	2
6	30	4	0	1	0
		8	2	2	3
	60	4	0	3	
		8	3	3	

* Three tubes were seeded on Petroff's gentian violet-egg medium in each case and the number designates the number of tubes upon which definite growth was found after four or eight weeks' incubation.

cultures, it was decided to compare the Petroff and Griffith methods, with slight experimental modification but adhering to the important factors concerned, on tuberculous sputums. In the Petroff method the washed

sediment was seeded, while in the Griffith method the latest suggested modification was adhered to, in that the entire mixture, without centrifugation, was seeded. The results are recorded in tables 3 and 4.

TABLE 2

Action of antiformin upon suspensions of tubercle bacilli in salt solution

TIME OF EXPOSURE TO ANTIFORMIN AT ROOM TEMPERATURE	STRENGTH OF ANTIFORMIN IN PER CENT ADDED TO EQUAL VOLUME OF BACILLUS SUSPENSION	TIME OF INCUBATION BEFORE READING TUBES IN WEEKS	CULTURE TUBES WITH GROWTH OF TUBERCLE BACILLI*					
			Gluckson		Human		Bovine virulent	
			Dorset's medium	Petroff's medium	Dorset's medium	Petroff's medium	Dorset's medium	Petroff's medium
2 minutes	10	4	3	0	0	1	2	3
	10	8	3	3	3	3	2	3
	20	4	1	1	0	2	3	3
	20	8	3	2	3	3	3	3
5 minutes	10	4	3	1	1	2	3	3
	10	8	3	2	2	2	3	3
	20	4	1	3	0	2	3	3
	20	8	3	3	3	3	3	3
10 minutes	10	4	2	1	1	0	2	3
	10	8	3	3	2	3	2	3
	20	4	0	0	0	0	3	3
	20	8	1	3	3	3	3	3
20 minutes	10	4	2	1	0	0	3	3
	10	8	2	2	2	1	3	3
	20	4	0	0	0	0	1	3
	20	8	2	3	3	1	3	3
30 minutes	10	4	2	0	0	0	3	3
	10	8	2	2	1	3	3	3
	20	4	1	1	0	0	3	3
	20	8	3	2	2	1	3	3
1 hour	10	4	0	0	0	2	3	2
	10	8	2	1	1	3	3	3
	20	4	0	0	0	1	0	3
	20	8	3	1	3	3	3	3

* Suspensions centrifugated after exposure to antiformin and centrifugate seeded. Three tubes were seeded in each case and the number designates the number of tubes upon which definite growth was found after four or eight weeks' incubation.

The results in table 3 indicate that the tubercle bacilli in microscopically positive tuberculous sputums, treated with an equal volume of sodium hydroxide solution up to 6 per cent concentration for thirty and

TABLE 3

Action of sodium hydroxide at 37°C. upon tuberculous sputum

STRENGTH OF NaOH IN PER CENT ADDED TO EQUAL VOLUME OF SPUTUM	TIME OF EXPOSURE TO NaOH AT INCUBATION TEMPERATURE	SPUTUM CULTURE RESULTS*			
		First Petroff's medium		Second Petroff's mediium	
		Growth	Contaminations	Growth	Contaminations
1	30	0	0	0	0
	60	1	0	0	3
2	30	0	0	0	1
	60	0	0	0	1
3	30	0	0	1	1
	60	0	0	1	1
4	30	0	0	0	0
	60	1	0	1	0
6	30	0	0	1	0
	60	2	0	1	0

* Three tubes were seeded in each case and the results are given as the number of tubes with growth after eight weeks' incubation.

TABLE 4

Action of antiformin at room temperature upon tuberculous sputum

TIME OF EXPOSURE TO ANTIFORMIN	STRENGTH OF ANTIFORMIN IN PER CENT	SPUTUM CULTURE RESULTS*			
		Petroff's medium		Dorset's medium	
		Growth	Contamination	Growth	Contamination
2 minutes	10	0	0	0	1
	20	0	0	0	1
5 minutes	10	0	0	0	0
	20	0	1	0	0
10 minutes	10	1	0	2	0
	20	0	0	0	0
20 minutes	10	0	0	0	3
	20	1	1	0	2
30 minutes	10	0	0	0	3
	20	0	0	0	0
1 hour	10	0	0	0	1
	20	0	0	0	3

* Three tubes were seeded in each case and readings made after 8 weeks' incubation, the results being indefinite at four weeks. The sputum was not centrifugated before seeding.

sixty minutes at 37°C., washed with physiological salt solution three times, and the centrifugated sediment seeded on gentian violet-egg medium, grow well. Up to the point of the addition of an equal volume of 4 per cent sodium hydroxide solution contaminations were fairly frequent, while above this the sodium hydroxide efficiently destroyed them. The results recorded in table 4 indicate that tubercle bacilli, in sputums treated with an equal volume of 10 and 20 per cent antiformin at 20°C. for two minutes to one hour and seeded on Dorset's egg medium and Petroff's gentian violet-egg medium, without centrifugation, grew well after twenty minutes' antiformin treatment in concentration as high as an equal volume of 20 per cent. Contaminations occurred, however, on Dorset's egg medium even after one hour's treatment with an equal volume of 20 per cent antiformin. On gentian violet-egg medium contaminations were less frequent and the preferred time seemed to be a ten minute exposure to an equal volume of 20 per cent antiformin.

Thus far no actual percentage figures of comparative value have been obtained, whereby one may practically judge the two methods, and all the data previously recorded must be considered more in the light of preliminary and introductory importance. It therefore seemed desirable to try to isolate the bacilli from a fairly large number of sputums coincidently and to obtain comparative figures by both methods. For this purpose a total of 54 sputums, 34 of them microscopically positive and 20 microscopically negative (the latter obtained from cases that had been positive microscopically before) were used for cultivating the tubercle bacilli coincidently by the Petroff method (slightly modified so as to use an equal volume of 4 per cent NaOH) and the final Griffith method of direct seeding without centrifugation. The latter was modified so as to use only the most satisfactory time interval and concentration of antiformin, as indicated by the above preliminary experiment, to avoid making the technique burdensome and thus defeating the important time-saving element of this procedure over the sodium hydroxide method. The results of this experiment are recorded in table 5.

It is to be noted that all of the 54 sputums had at some time before been positive microscopically, even though only 34 proved positive at the time of cultivation. After twelve weeks' growth in the incubator the cultures prepared from sputums, treated with an equal volume of 4 per cent sodium hydroxide solution, washed with saline solution and the centrifugate seeded on gentian violet-egg medium, revealed 14 pure cultures from 34 positive sputums and 6 pure cultures from 20 negative sputums,

as compared with 2 pure cultures from 34 positive sputums and 1 from 20 negative sputums after treatment with an equal volume of 20 per cent antiformin for ten minutes at room temperature and seeding direct without centrifugation. Of the total 54 sputums the sodium hydroxide and sedimentation method gave 35 per cent pure cultures, while the antiformin method without sedimentation gave only about 6 per cent pure cultures. This striking difference between the two methods is in all

TABLE 5

Comparison of sodium hydroxide incubator and antiformin room temperature methods for the cultivation of tubercle bacilli from sputums

SPUTUMS EXAMINED (54)	TREATMENT WITH EQUAL VOLUME	CULTURAL RESULTS*											
		After 6 weeks' incubation						After 12 weeks' incubation					
		+				−	Contaminations	+				−	Contaminations
		Total	3	2	1			Total	3	2	1		
Microscopically positive, 34	NaOH 4 per cent, 30 minutes	7			7	27	2	14	7	4	3	20	2
	Antiformin 20 per cent, 10 minutes					34	3	2	1		1	32	3
Microscopically negative 20	NaOH 4 per cent, 30 minutes.	5			5	15	4	6	3		3	14	4
	Antiformin 20 per cent, 10 minutes					20	1	1			1	19	1

* The results recorded are given as total positive cultures from the sputums as a single record and the approximate amount of growth obtained from 1 to 3 plus. The best growth on the 3 tubes only is recorded from each sputum, besides the contaminations resulting from each sputum by either method when seeded on 3 tubes of gentian violet-egg medium.

probability due to the fact that in the sodium hydroxide method the centrifugate is used for seeding, thus utilizing a larger and more concentrated number of bacilli. The suggested advantage of time-saving of the antiformin method, by eliminating washing and centrifugation, would seem, however, to be gained to the decided disadvantage of not obtaining pure cultures except in a comparatively small percentage of sputums which are positive microscopically. If this advantage is eliminated, as it seems to be from the above study, the sodium hydroxide method possesses a number of decided advantages. The reagent does

not deteriorate and can be kept much longer in soluble form and ready for use; it is easily prepared from an available dry, stock chemical; there is no necessity for seeding culture tubes at different intervals, thus shortening this to one manipulation; the price or time necessary for preparation of the antiformin is far greater than that of the sodium hydroxide; and the sodium hydroxide is more agreeable to work with. Barring these differences, the reagents seem to be equally desirable, though in different concentrations and at different time-intervals.

SUMMARY AND CONCLUSIONS

A comparison of the antiformin and sodium hydroxide methods shows the sodium hydroxide to be decidedly superior to the antiformin method. The advantages of time-saving and ease of manipulation possessed by the simplified antiformin method are gained at the expense of a decided decrease in the percentage of positive cultural results for human tubercle bacilli. If the sedimentation procedure is resorted to in both cases, which would seem to be highly advisable, the sodium hydroxide (Petroff's) method possesses the advantages of working with a more stable, more easily obtainable, less irritating and more economical reagent.

Suspensions of human and bovine tubercle bacilli in saline solution grew well after treatment, for thirty and sixty minutes, at 37°C., with an equal volume of sodium hydroxide up to 6 per cent concentration, or after treatment for two minutes to one hour at 20°C. with an equal volume of 10 and 20 per cent antiformin, while contaminators in sputum were about equally destroyed by an equal volume of 4 per cent sodium hydroxide for thirty minutes at incubator temperature, or by an equal volume of 20 per cent antiformin for ten minutes at room temperature, and seeding on gentian violet-egg medium.

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STUDIES ON THE RELATION OF MINERAL DUSTS TO TUBERCULOSIS

III. THE RELATIVELY EARLY LESIONS IN EXPERIMENTAL PNEUMOKONIOSIS PRODUCED BY CARBORUNDUM INHALATION AND THEIR INFLUENCE ON PULMONARY TUBERCULOSIS

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The third paper of this series will deal with carborundum dust and its effect upon the normal and the tuberculous guinea pig. The fact that so many employees are exposed to the inhalation of this dust in the manufacturing plants at Niagara Falls and the widespread use of carborundum abrasive products in grinding processes elsewhere make it desirable to determine, if possible, the biological reactions to this irritant and in particular to ascertain whether this, like some other compounds of silicon, increases susceptibility to pulmonary tuberculosis. The Carborundum Company of Niagara Falls, through its generous coöperation in furnishing the necessary amounts of the finest powdered carborundum has made this investigation possible.

No statistics are available to indicate the incidence of pulmonary tuberculosis among the employees in this industry. Winslow, Greenburg and Greenberg (12) in 1919 investigated the dust pollution of the atmosphere in two plants manufacturing abrasives and reported excessive quantities of fine particles of less than ten micra in diameter. Because of the hardness of this substance and because of the great numbers of fine particles which must be inhaled, they believed that there was "every reason to expect from analogy with other industries in which workers are exposed to a similar hazard, that dusts of this nature should be exceedingly deleterious to health." They found in the atmosphere of these abrasive factories a dustiness greater than that of the Joplin lead mines and concluded that "when one recalls Lanza's estimate that at least thirty per cent of the Joplin miners were suffering from

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industrial tuberculosis, it seems clear that the conditions existing in the abrasive factory constitute a grave menace to the health of the persons employed therein."

In 1918 the U. S. Public Health Service conducted an investigation of the hazards in the industries at Niagara Falls, New York, "the idea being to get the facts concerning hygienic conditions quickly and at the same time to get practical results in the way of improving working conditions for both men and women workers." A report of this investigation was published by Holmes (10) in 1920. As to the effect of carborundum dust on lung tissue, Holmes says

> with the above characteristics (very hard, chemically inert, jagged particles) in mind, it is easily understood why working in a polluted atmosphere should injure the delicate lung membranes, producing respiratory affections of various kinds and acting in a contributory manner to the onset of other diseases not directly due to dust inhalation. The dust is not eliminated from the lungs, but once it is inhaled it stays in the lungs without a chance of removal by coughing or through the lymphatic system. The effects of the inhalation of this type of dust appear slowly and take a comparatively long time to develop (one to five years).

No mention is made in this report as to what type of respiratory affections are prevalent among the employees exposed to this dust.

Such conjectures are based purely upon the hypothesis that a dust is injurious because of its physical properties. The English investigators, Haldane (9), Mavrogordato (11), Collis (4), and Gye (8), are emphasizing the chemical composition of dusts as an important, and even predominant, factor in determining their irritating qualities. Experience has shown that silicon compounds, in the form of the dioxide, or silica, are preëminently pulmonary irritants. Experiments with such dust in the form of granite have already been reported (7). Carborundum, being a nonsilicious compound of silicon, offers an opportunity to observe the behavior of silicon combined in a different manner. Having in mind the desirability of testing this chemical theory of the action of dusts and at the same time wishing to investigate the effects of carborundum dust *per se*, this experiment was undertaken.

As far as has been ascertained, Beattie (1) is the only one who has studied experimentally the effect of the inhalation of carborundum. His work has been reported in a hearing before the Royal Commission on Metalliferous Mines and Quarries with the result that, while it is

possible to obtain from the minutes of this hearing a general impression of his experiments, the technical details of experimentation and its results are extremely difficult to evaluate. He exposed normal guinea pigs in a dusting box, so constructed that they were compelled to inhale excessive quantities of carborundum dusts. Its concentration was not measured, but the atmosphere in the box was nearly saturated. The daily exposures never exceeded three hours and the animals from which he drew his conclusions had received a total exposure of twenty-one and sixty-eight dust-hours, respectively. In the minutes of the hearing he says

. . . . . now I take carborundum. This is a short exposure of only twenty-one hours and this shows very obviously the immense change which has taken place; the great amount of dust, the whole thing being absolutely black, and, again, specially concentrated toward the surface. You will see in the next photograph that you have it however right through the lung. You have this very definite fibrosis and the obliteration. . . . . You still see the outline of some of the cells and the fibrosis. Here you have a later period of sixty-eight hours in which you have part of the lung tissue quite obliterated with nodules passing into the lung in all parts. (Chairman) that is interesting on the ground of it not being a silica?—Yes. The dangerous ones you have shown us have all silica at the base of them?—Yes. And here is carborundum which is not?—Yes.

From studies of cases of human pneumokoniosis Beattie believed that in areas of excessive fibrosis the blood supply is diminished or entirely absent, due to the obliteration of the blood vessels, and that the consequent damage so lessens the resistance of the tissues that they are especially susceptible to implantation of the tubercle bacillus.

The Carborundum Company has collected data from various sources which have been published under the title, *Chemical and Physical Properties of Carborundum* (3). Quoting from this pamphlet:

Carborundum is a chemical compound composed of one atom each of silicon and carbon. Its chemical name is silicon carbide and its chemical symbol SiC. A typical analysis of a high-grade product is as follows:

Si (combined)	69.60
C (combined)	29.40
Fe	0.10
Al	0.45
Si (free)	0.25
Ca	0.20

The crystalline product is decomposed at temperatures from 2220 to 2240 degrees C. There is no oxidation in pure oxygen at 1000 degrees C. Carbon dioxide and hydrogen dioxide only become active at their dissociation temperature of 1775 or 1800 degrees C. Crystalline carborundum as it comes from the furnace often shows a brilliant play of iridescent colors. Its hardness is between that of the ruby and that of the diamond, or between 9 and 10 on Mohr's scale. Its specific gravity varies from 3.12 to 3.20 and the "carborundum Grain No. F.," the finest powdered product (*used in this experiment*), contains 119 pounds per cubic foot.

In the experiments to be reported, guinea pigs have been exposed to an atmosphere of carborundum dust in a chamber constructed on the plan of that described and illustrated in the first paper of the series (7). Mr. Morris Dworski has made determinations of the dust concentrations in the atmosphere in this chamber with a Palmer apparatus and he has also made a series of measurements of the dust particles. Micrometer measurements of the dust as received from the manufacturer show a range, in maximum diameter of the particles, of from 48 to 0.3 μ. Of those particles fine enough to remain suspended in the air, about 75 per cent are 1.5 μ or less in diameter. The following tabulation represents average dust counts of four 60 cubic foot samples taken with the Palmer apparatus, the intake being placed in the vertical centre of the machine and the rate of sampling varying from 2.7 to 3 cubic feet per minute:

Number $\frac{1}{4}$ Standard Unit particles per cu. ft. air (Magnification 85)	Number $\frac{1}{4}$ Standard Unit particles per cu. ft. air (Magnification 410)	Number particles under 1.5 μ in diameter, per cu ft. air	Mgm. dust per cu. ft. air
33,430,000	135,208,000	101,778,000	4.88

($\frac{1}{4}$ Standard Unit is a particle the diameter of which is $10 \times 10\ \mu$ or less.)

For a discussion of these figures and their derivation the reader is referred to the second paper of this series on the inhalation of marble dust (6). It is of interst to compare these concentrations with those recorded by Winslow, Greenburg and Greenberg (12) in their investigation of the dust hazard in the abrasive plants at Niagara Falls. A summary of their figures, together with the average counts obtained by Mr. Dworski, is as following:

PLACE OF SAMPLING	½ STANDARD UNIT PARTICLES PER CU. FT. AIR	WEIGHT TOTAL DUST MGM. PER CU. FT. AIR	PERCENTAGE OF INORGANIC SOLIDS
Abrasive Factory B: Lathe room, average.....	4,006,000	1.039	96.4
Abrasive Factory B: Shaving room, average....	113,930,000	3.114	93.5
Abrasive Factory B: Grinding room, average....	14,451,000	1.350	73.4
Abrasive Factory C: Grinding room, average....	71,718,000	6.105	98.5
Abrasive Factory C: Coke grinding room, average	669,000,000	21.50	70.4
Experimental dusting chamber average.....	33,430,000	4.88	97.16

A comparison of the last group of figures with those which precede it indicates that the dust concentration used in this experiment, while not equivalent to the heaviest encountered in these industrial operations is, nevertheless, of the same general magnitude of that in some rooms of the manufacturing plant.

Eighteen normal guinea pigs of an average weight of 450 gm. were infected by the inhalation method (2) with a moist spray of R1, a culture of tubercle bacilli of low virulence. On the same day these animals, together with 18 normal ones of the same average weight, were placed in the dusting chamber and exposed thereafter eight hours daily, over a period of seven months, to the inhalation of carborundum dust in the concentration described above. In this period the total exposure amounted to 1599 dust-hours.

For the first month after the dust inhalations all the animals lost weight and appeared ill-kept, but they showed no symptoms. After becoming accustomed to the dust they steadily gained in weight and regained their usual normal appearance. Only two animals of the entire series died, one of endemic chronic pneumonia and the other of tuberculous pneumonia. Two others were killed during the first month because of symptoms of chronic pneumonia; autopsy confirmed the clinical diagnosis. Nineteen animals were killed in pairs or groups of four at monthly intervals during the course of the dust inhalation; the surviving 13 were sacrificed at like intervals after the cessation of the exposure. At the end of sixteen months two controls and three tuberculous animals survived. They were apparently well and had gained in weight, the controls showing an average gain of 487 gm. and the infected animals 310 gm. With their death the experiment was completed. As controls to the infection the same series of fourteen animals served as for the previously reported marble and granite experiments. All technical procedures were the same as described in the previous papers (7) (6).

EFFECTS OF CARBORUNDUM DUST INHALATIONS ON NORMAL ANIMALS

In gross, the surface of the lungs of the dust control animals showed from month to month a progressive increase in subpleural dust deposits which attained a maximum at the cessation of the exposures and showed little alteration thereafter. The deposits were first noticed at the end of one month and consisted of small, irregularly mottled, greenish-black patches located at the tips of the cephalic lobes. With the lapse of time more and more of the surface of these lobes became involved, as did the ventral lobes where the first deposits occurred in the paravertebral regions. From these points the pigmentation spread, until the whole pleural surface was more or less evenly involved. The tracheobronchial lymph nodes contained small amounts of pigment at the seventh month, but from then on until the end of the experiment each succeeding animal showed more dust in this location. The pigmentation was never sufficiently great to be visible on the surface of the node; only on section could irregular patches of color be detected. No pigmentation was ever seen in the mesenteric lymph nodes or other viscera. In the tuberculous animals, in addition to the diffuse pigmentation, there were heavy deposits of dust about individual tubercles. Dust reached the tracheobronchial lymph nodes at the same rate as in the control series.

On section there were diffuse deposits in all parts of the lung. Cleared sections, prepared by the Spalteholz method, of the lungs of animals autopsied after the cessation of the exposures, showed a rather definite tendency for some of the dust to collect in relatively immobile structures like the sheaths of blood vessels and bronchi, beneath the pleura, and about tubercles. But in all the lungs there remained very extensive, diffuse deposits, and in no case was there any evidence of the mass mobilization of large amounts of dust-filled phagocytes and the consequent clearing of wide areas which was such a characteristic picture of the lungs of the granite series.

When thin paraffin sections were studied, dust was found in almost every alveolus, occasionally as free particles, but usually contained in phagocytes. These cells presented their usual characteristics; at first they contained only one nucleus, but later two or more were frequently encountered. In the late stages the cells were often so completely filled with heavy black dust that the nucleus was more or less obscured. The tendency to plaque formation by fusion (?) of several

phagocytic cells, although occasionally present, was not nearly as marked as in the granite animals. While the main mass of the dust remained scattered throughout the air spaces, small collections, contained in wandering phagocytes, were embedded in the areolar coats of the bronchi and blood vessels. The subpleural collections, when studied serially, were not collected about any particular structure but simply lay in a group of apparently normal alveoli. In the lumina of certain bronchioles, there occurred a small amount of exudate, consisting of polymorphs and dust; when studied serially this exudate was not found to have its origin in an inflammatory focus elsewhere in the lung. Such intrabronchial collections were found in animals with no trace of pneumonia, tuberculosis or other infectious process. As such exudate was found in animals killed after cessation of the dust exposures, it was thought that some elimination by the bronchial path could occur.

The reaction to these extensive and persistent deposits of dust in the lungs of the control animals was surprisingly insignificant. In a group of alveoli, the lumina of which were practically occluded by heavily laden phagocytes, the alveolar walls showed only a very moderate thickening which was due to infiltration of plasma cells and endothelial leucocytes. Proliferation of fibroblasts and fibrosis did not occur; a connective tissue stain showed absolutely no increase in collagen. After five months of irritation the bronchi and bronchioles lined by cylindrical epithelium reacted with an oedema and a lymphocytic infiltration of the muscular and areolar coats, but this reaction subsided immediately upon discontinuing the irritation. This reaction was quite marked, so that the swollen mucosa was thrown into deep folds, but acute exudation into the lumen was absent. It occurred in all 5 controls and in 5 experimental animals killed between the fifth and the tenth month.

EFFECTS OF CARBORUNDUM DUST INHALATIONS ON TUBERCULOUS ANIMALS

The course of a primary tuberculosis following the inhalation of the low virulent strain of tubercle bacillus R1 underwent the most profound alteration as a result of the inhalation of carborundum dust during its development. No indication of such an alteration occurred as the result of the inhalation of granite dust, although in that experiment the animals were not allowed to live after ten months. In animals exposed to carborundum dust, tubercle formation occurred in the usual time, manner and location; discrete subpleural tubercles with well developed

caseous centres were present one month after infection. Dust appeared in all parts of the tubercle, from the caseous centre to the periphery. When caseation occurred, large deposits, freed by the disintegration of the bodies of the phagocytes, remained in the debris. These tubercles differed from those in the infection controls in that the zone of granulation tissue about the periphery was much thicker and contained heavy deposits of dust. Phagocytes loaded with pigments were scattered everywhere, singly and in groups. Dust of this type, occurring in a location where fibroblast proliferation has already been initiated, is apparently able to continue the process. As a result, a heavy fibrous mantle was formed about each tubercle which interrupted the usual process of resolution (5) which should be completed in from six to eight months. Some tubercles undoubtedly did resolve, as the later animals showed fewer lesions than those killed early; but throughout the dust series, in the lungs of all infected animals, even in the three killed at sixteen months, there were encountered heavily pigmented subpleural scars, containing masses of inspissated caseous matter which represented persistent primary tubercles. In the infection-control series such tubercles had completely resolved and disappeared by the seventh month, whereas in the experimental animals tubercles with caseous centres were still present sixteen months after infection.

Calcification in the caseous centres of tubercles was encountered in two of the animals last killed. It was frequent in the marble series and has been seen in only one other animal (6).

A similar persistence of primary foci was encountered in the granite series, and apparently the explanation in each case is the same; namely, that the dust is capable of continuing a connective tissue proliferation once it has been set in motion by the action of the tubercle bacillus. But when either dust alone is in contact with the uninjured lung tissue the connective tissue response is very slow or entirely absent.

The most unusual feature encountered in these animals was the uniform tendency of the primary tubercles to spread and thereby result in progressive pulmonary tuberculosis. Only in one or two isolated instances during several years of constant work with this strain of tubercle bacillus has such a tendency been observed. In such cases no explanation could be offered. In the present experiment all of the dusted animals killed after the fifth month showed definite evidence of autogenous reinfection of the lungs. The first evidence of spread consisted of focal collections of proliferated endothelial cells gathered about the distal ends of certain

ductuli alveolares. These nodules frequently contained carborundum particles, but tubercle bacilli could not be detected, as is often the case in allergic animals. Along with this nodule formation exudative phenomena, such as accumulations of polymorphs and eosinophiles, were often seen. At the same time the adjoining alveolar walls underwent proliferative changes, so that there resulted a more or less dense mass with partial obliterations of the normal architecture. A connective tissue stain, however, very clearly demonstrates the fact that the normal alveolar outlines had been obscured by an overgrowth of fibroblasts with an excessive collagen formation, which encroached upon the cell accumulations within the alveoli. In this instance a definite fibrosis was in process of formation.

These original secondary foci suffered an inflammatory softening (which was not caseation), the central portions became necrotic and an exudate of polymorphs and fibrin collected within them. As a result, for a second time dust particles were freed in large numbers from the bodies of phagocytic cells. Nearby bronchioles were found to contain an exudate of polymorphs and mononuclear cells with large amounts of intracellular dust. In most cases, after prolonged search, a few tubercle bacilli were found, and in one instance (the animal dying of tuberculous pneumonia at seven months) enormous numbers of acid-fast bacilli were present in the bronchial exudate.

By studying a large number of serial sections with the preparation of reconstructions made according to the method of Adolf Meyer (tracings on glass plates which were then superimposed), it was possible to demonstrate that material from such necrotic foci was aspirated into fresh portions of the lungs with the establishment of new foci of disease. All of the animals, eight in number, killed in the interval from three to seven months, showed the earlier stages of this process. One guinea pig died at seven months of tuberculous bronchopneumonia, a terminal stage of the same process, while the five guinea pigs killed from the tenth to the sixteenth months showed more or less advanced foci of the same type. In four cases one or more lobes were completely involved. Ulcerations of the bronchial walls, with the establishment of new foci of dissemination, were frequent.

Although the mechanism by which the primary focus, due to the presence of dust, is enabled to liberate bacilli and thereby produce an autogenous reinoculation of the lung cannot be absolutely determined, we may imagine that the steps are somewhat as follows: When the

primary focus caseates, dust is freed in appreciable masses from the bodies of the phagocytes; being free, this dust exerts an attraction (?) for new phagocytes, which make their way into the caseous zone to engulf these uncared-for particles. In picking up carborundum particles, tubercle bacilli are also taken up by the same cell. This step is purely hypothetical, for at this stage the acid-fast bacilli in the caseous focus can no longer be demonstrated by the Ziehl-Neelsen stain. Bacilli and dust are carried by the phagocytes through the air passages to that danger zone, the distal end of the *ductulus alveolaris*. Here a new focus is established upon allergic soil and we find evidence of inflammation as well as proliferation. With the softening of this second depot, bacilli and dust are again freed; they are now in a position where they readily find their way into a bronchus, through which aspiration and the establishment of remote foci readily occur. Such an hypothesis offers an explanation which, in general, is in accordance with the observations, but the most important link, the demonstration of bacilli in the primary focus, is lacking. Other experiments made in the laboratory have demonstrated, by inoculation, the presence of viable tubercle bacilli in such caseous foci when staining methods absolutely failed.

The intrapulmonary lymphoid tissues, both peripheral and central, in the dust controls and in the infected animals, underwent hyperplasia, which was entirely similar in both series. In only two isolated instances was there any extensive tuberculosis in such tissue within the lung. The lymphatic vessels appeared to be wide open and there was no evidence to suggest an interference with the lymphatic drainage. In the outer walls of every primary tubercle in which more or less fibrosis had occurred there were large gaping lymphatic vessels offering every opportunity for the escape of bacilli. Some bacilli did undoubtedly escape and passed directly to the tracheobronchial nodes, as there was no evidence of an interruption of the usual course of the tuberculous process in these nodes.

The tracheobronchial lymph nodes in both the dust-control and the infected series showed microscopic dust deposits at a somewhat later date than did those of the corresponding granite series; at two months an occasional particle could be found, but it was not until the fourth month that any considerable quantities of dust had collected. From then on, until the sixteenth month, there was a steady increase in the deposits in both series. In the last five animals killed very large quantities were contained in all parts of the nodes. In the two dust

controls of this period there was a very definite and somewhat extensive *fibrosis* which had obliterated many of the medullary sinuses. This reaction may be explained as the result of concentration of excessive amounts of the irritant within a small focus. In the infected series the endothelial cells forming the tubercles usually contained many fine dust particles. Fibrosis dominated the picture in these tubercles, especially in the later animals of the series.

The involvement of the pleura in this experiment has been of great interest and has suggested some difficult problems. In 50 per cent of the dust-control series and in 72 per cent of the experimental animals, exclusive of any focus of endemic pneumonia, there has been more or less extensive fibrosis in the pleura. In the controls the process was usually localized in small areas, but in the infected animals pleural adhesions frequently covered the entire surface of a lobe. Interlobar pleurisies were frequent. Lungs in which this reaction was most severe also showed some tendency to peribronchial and perivascular fibrosis although this was not marked. Dust was absent from the pleura itself and occurred only in the subjacent alveoli. It is possible that this reaction was the result of a previous chronic organizing pneumonia, but in no other group of animals has healing of such a process been observed to this degree. And again, primary inhalation infection is followed by the formation of a series of subpleural tubercles which are covered by a cap of granulation tissue (5). These were marked in the present experiment; but the secondary foci arising from the extension of the disease usually involved many alveoli immediately beneath the pleura. Yet in no case did these secondary foci excite the slightest response in the pleura. Discussion of these phenomena is reserved for a future communication.

Examination of the abdominal viscera showed no evidence of dust in any organ or lymph node, yet the color of carborundum particles is so pronounced that very minute deposits are readily detected. Isolated tubercles of microscopic dimensions were detected three times in the spleen and once in the liver. Such sporadic occurrence of microscopic tubercles below the diaphragm is characteristic of inhalation infection with the tubercle bacillus. When animals are infected by this method with virulent bacilli generalized macroscopic lesions of the abdominal viscera are the rule. The character of the abdominal tubercles in these animals is conclusive evidence that the carborundum inhalation has in no way affected the virulence of the bacillus, and suggests that the secondary invasion of the lungs is due to local mechanical effects.

A consideration of the observations which have been presented must convince the most casual reader that, under the conditions of this experiment, carborundum dust is a dangerous irritant to pulmonary tissues. Application of these results to human beings exposed to the inhalation of carborundum dust should be made with caution. While the human lung may not be regularly infected with attenuated tubercle bacilli, it nevertheless usually harbors some more or less healed foci of tuberculous infection. If the suggested phagocyte theory of the mode of spread be correct it would be possible for such a mechanism to be active in causing extension from an imperfectly healed focus in the human lung, provided that focus were established during a period of exposure to dust. But this hypothesis is based upon the study of a small number of guinea pigs' lungs, and biological analogies from one species to another are notoriously dangerous. Material has already been prepared for the study of subcutaneous infection, superinfection and infection with virulent tubercle bacilli in animals which have been exposed to the inhalation of carborundum dust. When this material has been studied and analyzed further applications may be possible. But such evidence should be controlled by a thorough study of autopsy material in persons exposed to the inhalation of carborundum dust.

SUMMARY

As a result of this study certain factors in the biological reaction of the normal and of the tuberculous animal to this irritant have been established. When this dust is inhaled it is widely distributed throughout the parenchyma of the lung. Once deposited, it tends to remain *in situ;* but there is a tendency to focalization beneath the pleura and in the walls of relatively immobile structures like the sheaths of blood vessels and bronchi. Although relatively small amounts are removed by way of the bronchi and by the lymphatic system to the tracheobronchial lymph nodes, it seems improbable, from the evidence at hand, that the lung would ever be able to rid itself of the major portion of the dust. Prolonged daily exposures finally cause an oedematous condition in the bronchi and bronchioles which, however, rapidly subsides when the irritation is discontinued. The reaction of the normal lung to excessive quantities of this irritant is surprisingly insignificant. A true fibrosis in the parenchyma of the lung has never been observed. In the lymph nodes, on the other hand, a definite fibrosis was frequently encountered. In the lung, in which carborundum inhalation is con-

tinued during the course of development of an inhalation tuberculosis, *extensive fibrosis is common.* The most remarkable result in this experiment has been the alteration in the course of the tuberculous process induced by the inhalation of this low virulent tubercle bacillus; from a self-limited process, which usually heals by resolution, it has been transformed into a progressive tuberculous bronchopneumonia. As a basis for this transformation it has been suggested that, through caseation of the primary focus, excessive amounts of dust have been liberated which attract new phagocytes to the area. By the agency of these phagocytes tubercle bacilli are accidentally transported to uninvolved portions of the lung and fresh foci of infection are established. Two other possible explanations have been suggested, one the mechanical obstruction to the drainage from the lung as the result of fibrosis about the lymphatic vessels; and the other, a direct effect of the dust upon the infecting organism. Opposed to the mechanical explanation is the observation that no fibrosis is present during the early stage of extension; later, when a generalized tuberculosis has developed, this factor may play some part. The argument that the bacillus may have been altered is met with the fact that there has been no serious extension to the abdominal viscera, such as regularly occurs when virulent bacilli are implanted in the lung tissue.

In so far as proliferation of connective tissue may be considered as evidence of the ability of a mineral dust to injure pulmonary tissues, carborundum dust is apparently harmless to the normal lung, at least within the time limits of this experiment. If mere hardness and sharpness of a dust particle were sufficient to provoke the fibroblastic proliferation characteristic of pneumokoniosis, carborundum dust should satisfy these conditions, for its hardness is between that of the ruby and the diamond, and its abrasive qualities are well known. The failure to produce this response in the lung may be considered negative evidence, and favoring the chemical theory of the action of dust which has been advanced by the English observers. As carborundum does not contain silicon in the crystalline form of silica, they would assume that it would not produce a silicosis. The mechanical hypothesis, suggested in explanation of the mechanism producing extension of partially healed tubercles, requires further investigation and will be controlled by the employment, in a similar manner, of other nonsilicious dusts. However, it should always be borne in mind that silicosis in the human lung requires many years for its development and, while no hint of a proliferative process has been detected in the normal guinea pig's lung, yet such a reaction might be produced if animals could be exposed for longer periods.

The evidence for the solution of these problems is not to be found in such experiments as have been reported in this series of papers. These we have written more in the nature of preliminary communications than as finished results. They have served to determine the problems before us and to open up lines of investigation. Other work of a more fundamental character has been in progress for some time which, it is hoped, may illuminate the problem of the action of mineral dusts on pulmonary tissues.

In conclusion I wish to express my acknowledgment to the officers of the Carborundum Company for their interest and for their gifts of large amounts of carborundum dust; to the U. S. Public Health Service for the loan of a Palmer apparatus; and to Mr. Morris Dworski for determination of the dust concentrations and his measurements of the dust particles.

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CAUTERIZATION

ITS EFFECT UPON CUTANEOUS TUBERCULOUS LESIONS AND THE DISSEMINATION OF TUBERCULOSIS IN THE GUINEA PIG

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Cauterization, in some form or other, has been practised so long that its inception cannot be traced. To cite the uses to which it has been put and the diseases in which it has been tried would require a resumé and history of almost the entire field of surgery. Moodie (1) studied lesions among primitive and ancient peoples due to cauterization and cites Manouvrer, a French anthropologist, who studied a curious lesion, since known as the *sincipital T*, on skulls bearing marks of cauterization of the scalp from the Dolmen de la Justice at Eponé, near Mantes on the Seine River, Seine-et-Oise in France, and dating to the Neolithic period (3000–7000 B.C.). Buchtel (2), writing in 1919 on the use of the cautery, states that "many volumes have been written on hydrotherapy; the literature on calloritherapy is meager." He gives three indications for its use: first, in malignant tumor; second, to prevent infection; and third, to prevent hemorrhage. Buchtel enumerates its use in benign and malignant tumors of the jaws (Bloodgood and Ochsner), carcinoma of the cervix (Percy), breast (Judd, Greenough, Lyle and Mayo), and bladder (Squier), abscess of the lung and abdominal surgery, gonorrhoeal leucorrhea (Kelly), noma, war wounds (Moynihan), nevi and certain nasal operations.

Whether or not it was used to any extent before the twentieth century in treating tuberculosis is difficult to state, since literature bearing directly on this subject is meagre and of purely clinical nature, and modern American textbooks on tuberculosis fail to mention it. As a matter of interesting historical record Sonnenburg (3) in 1891 used the cautery upon tuberculous cavities in the lung of man, puncturing through the pleura into the cavity, removing the pus and then opening the small cavities one after the other, until the entire field was converted into one large space. As evidenced by this report, it is fair to assume that,

with the passing trials of almost all available surgical and medical procedures in tuberculosis, cauterization should have had its transient advocates whose enthusiasm could not be aroused sufficiently to leave an indelible impression or a written record of sufficient note to be easily accessible.

In laryngology, however, the actual cautery has, within the past century, attracted some attention for its possible value in the therapy of laryngeal tuberculosis. In 1867 Voltolini (4) used the cautery for laryngeal tuberculosis, in a man with advanced pulmonary disease, but the extent of the infiltrations in this case so discouraged him that he discontinued the method. Mild interest in the method then existed, until 1907, when Grünwald (5) published a monograph on the subject, in which he elaborately described the technique, formulated the indications for its use and gave the results of its use on man. He believed it to be the only serviceable method in widespread and deep infiltrations of the larynx. In 1911 George B. Wood of Philadelphia reported experimental work with the actual cautery in the treatment of localized tuberculosis (6). In introducing his report he states that

> for some time I have felt that the actual cautery, and preferably the platinum wire heated by electricity is by far the most effective method of combating tuberculosis of the upper respiratory tract. The striking results which have followed its use in cases of pharyngeal tuberculosis have confirmed my belief in its efficiency. If a pharyngeal lesion is not too extensive and can be reached by the heat, I am of the opinion that if treated with the cautery it will in all cases heal promptly. . . . . The good results following the use of the cautery are equally great in the larynx, provided the lesion is so placed as to make it accessible to the action of the heat. The beneficial action of the cautery seems to reach distinctly beyond the area of destruction, and the resulting scar does not appear to be easily reinvaded.

Besides his clinical experiences Wood reports studies on guinea pigs, on the histological appearance of a cautery wound made through a tuberculous focus. Attenuated human tubercle bacilli were inoculated just beneath the skin of 3 guinea pigs, each animal being inoculated on each side of the abdomen, so as to make two distinct and separate lesions. In guinea pig 1, three-quarters of one lesion was cut out and about half of the other was destroyed with the electric cautery. One month after infection the lesion that had been cut six days prior to autopsy showed progression macroscopically, while the burnt lesion appeared to be

healing. Both lesions in guinea pig 2 were cauterized and three days later (about 6 weeks after infection) the eschar was still adherent, there seemed to be a slight shrinking of the tuberculous area, and the inguinal lymph nodes were enlarged and caseous. Both lesions and an enlarged right inguinal lymph node were cauterized in guinea pig 3 about 6 weeks after infection and the animal was killed about 3 weeks later. Macroscopically, the lesion on the left side of the abdomen showed a healthy, shallow, raw surface without induration, which probably was caused by the breaking of a partially healed wound through rough handling. The wound on the right side was so nearly healed that it was difficult to find it until the hair had been removed, while the inguinal lymph node showed a small, healed, punctate scar, but it was so completely caseous that the histological study did not show anything of interest. Wood concluded that

these few experiments seem to have substantiated certain clinical observations as regards the use of the cautery in treating localized tuberculosis. There is no doubt that a retarding influence is exerted beyond the area actually destroyed by the heat. . . . . Anything that aids in the formation of fibrous tissue aids in the cure of the disease. Further, by the induction of acute inflammation with the subsequent formation of new blood vessels, nutrition is brought to a part which would otherwise break down into a necrotic mass because of a lack of sufficient food to nourish the tissues. Again, the eschar produced by the burning prevents reinoculation until the tissue has become sufficiently resistant to protect itself. Also, the cauterization seals the lymphatics and blood vessels, thereby preventing a spread of the disease either locally through the lymph system or generally through the blood vessels.

In 1922 Wood (7) reported further on the same subject, but gave mainly clinical information. Regarding the purpose of the treatment he states, "The local treatment has two purposes: one palliative, applicable especially to advanced and hopeless cases, and the other curative." In this paper he dealt only with the question of curing laryngeal tuberculosis and further stated that

the real curative measures belong to the domain of surgery. Bloody surgery of the tuberculous larynx, though frequently accompanied by very favorable results, has reached the end of its usefulness for the sole reason that the electric cautery will in almost every instance accomplish the same results, with none of its disadvantages, and it does exceedingly more than could ever be hoped for from other surgical procedures. It is undoubtedly the method *par excellence*

for the treatment of laryngeal tuberculosis, and its use brings to the surgeon that peculiar sense of elation which he feels when, through his interference, suffering and death have been averted. . . . . I believe that the beneficial action of the cautery is due more to the revitalization of the tissues than to the destructive action of the heat.

The object of the cauterization is the production of an eschar rather than destruction of all the tuberculous tissue. The instrument and clinical technic are fully described in this article.

In passing, mention may be made of the use of the cautery by Jacobaeus (8), for the purpose of removing tuberculous pleural adhesions. As a result of an extensive study on man, covering a period of ten years, it is concluded that string-shaped adhesions, up to the thickness of a little finger, or membrane-shaped adhesions, can always be burnt off without great risk, while with surface adhesions there is need for great precaution. In cauterizing the lungs, themselves, there is the risk of hemorrhage, as well as that of opening tuberculous foci and cavities, with consequent infection of the pleura. Although this work does not bear directly upon the subject of cauterizing actual tuberculous foci, the effect upon the local lesion *per se* or upon the dissemination of the disease from this focus following cauterization, it gives us some information upon the use of this procedure in destroying adhesions and fibrosed lesions formed as a consequence of tuberculosis. Just how often viable bacilli may be present in these lesions also remains to be accurately determined, as well as the effect of the cautery upon them, although Jacobaeus has studied this to a certain extent.

Pertinent to any study on the spread and development of tuberculosis in guinea pigs is the study on this subject by Krause (9) in 1920. The significant points brought out by this study were that, following subcutaneous infection, tubercle bacilli follow a definite course, from the local inguinal to the superficial inguinal nodes, common iliac nodes, the plexus and nodes of the abdominal aorta, the thoracic duct, into the venous system and then into the lungs, tracheobronchial lymph nodes, spleen, liver and other organs. The evolution and spread of macroscopic changes from the point of inoculation is also well described. Beginning at 6 days, distinct changes are to be noted in the superficial inguinal nodes; at 12 days the nodes are "enlarged," opaque and with yellowish spots" (necrosis); at 18 days there is abscess of the soft tissues and "proceeding cephalicwards a chain of five to seven enlarged nodes,

of which the one next to the abscess is largest;" at 6 days the iliac node is "somewhat enlarged," while at 18 days it is "as large as a bean and necrotic;" the first time the spleen can be called grossly tuberculous is at 12 days, when it may show small yellow tubercles, and at 18 days and afterward lesion is always well established; the paraäortic nodes were noticeably enlarged from 12 days on; the lungs never exhibited gross tubercle,—up to 26 days; the first time the tracheobronchial nodes were unquestionably grossly tuberculous was at 22 days. After large subcutaneous injections the method of tissue inoculation revealed that the bacilli are carried centralward by the lymphatic stream and thence into the blood throughout the body with great facility and rapidity; at four days and earlier they have reached the iliac node of the inoculated side by way of the lymphatics; at four days or perhaps earlier they are present in the spleen; at four days they are also in the lung and tracheobronchial nodes; at any given time during the period of from four to nine days after infection there are more bacilli in the iliac node of the inoculated side than in the spleen or lungs or tracheobronchial nodes. These findings by Krause were corroborated by Rensch and Moore (10) in a study on an elaborate series of infected guinea pigs. They point out that the macroscopic tuberculous involvement follows a definite organ sequence which is related to the time interval and can be correlated fairly well with the dose of bacilli (human) used for infection.

In view of the above encouraging studies, though only few in number, by Wood on the value of cauterization in localized tuberculous lesions, and the availability of standardized experimental data on guinea pig tuberculosis contributed by Krause, and Rensch and Moore, it was deemed advisable to study, in a large series of guinea pigs infected intracutaneously, the effect of cauterization, by means of the electric cautery, upon the local lesion itself and upon the dissemination of the infection from this local focus, utilizing for this purpose bacilli of low and high virulence and graded infecting doses of wide range, so that all possible effects of the cauterization upon the tuberculosis would be revealed in the study. In the first experiment avirulent human tubercle bacilli were used, so that deductions could be drawn regarding the effect of the cauterization upon a purely localized lesion produced by living bacilli, and to determine whether the virulence of the bacilli or spread of the disease from the local intracutaneous site was in any way enhanced by the cauterization. The bacilli used were an old laboratory

strain of human tubercle bacilli, which originally was able to produce a generalized tuberculosis of guinea pigs after subcutaneous inoculation, in doses as small as 0.000,001 mgm. within two months after inoculation. After more than ten years of artificial cultivation it has become incapable of producing more than a local lesion in guinea pigs, within two months after subcutaneous inoculations of 1 mgm. In order to more accurately observe slight effects upon the bacilli or the spread of the disease from the local site, and especially the effect upon the local lesion itself, the intracutaneous inoculations were made in three different amounts, namely, 1, 0.1 and 0.001 mgm., always in a uniform amount of suspension, 0.1 cc., throughout the experiment. To note the effects of the cauterization at different periods after inoculation, the lesions or, in the absence of these, the sites of intracutaneous inoculation were carefully but efficiently removed by the cautery, immediately after injection, one, two and four days, one and two weeks, and one month thereafter. The local intracutaneous site was marked to a point slightly beyond the extent of the small bleb formed by the inoculation of the 0.1 cc. of fluid, and was then efficiently and completely cauterized to this point at the designated time. The results of this experiment are recorded in table 1.

An examination of table 1, in which are given the graded findings of both the local lesion and the generalized dissemination of the tuberculosis from this focus, reveals that the cauterization of a local intracutaneous tuberculous lesion, produced by avirulent human tubercle bacilli (simulating experimentally the nonprogressive clinical type of local tuberculosis), efficiently destroys and has a beneficial influence upon this local lesion, without materially tending to in any way influence the tendency to systemic dissemination, regardless of whether cauterization was performed immediately or one month after the initiation of the local intracutaneous tuberculous lesion.

That conditions, may, however, be entirely different when cauterizing a tuberculous lesion, produced by virulent bacilli, seemed likely and worthy of study; and for this reason the above experiment was repeated, but, instead of using the avirulent human tubercle bacilli, a highly virulent human tubercle bacillus, "Gluckson," recently isolated from sputum by Petroff's method and capable of producing a generalized tuberculosis in guinea pigs within two months, following subcutaneous inoculation of 0.000,001 mgm., was used for the intracutaneous inoculations. The latter ranged from 1.0 to 0.000,000,01 mgm., and the local site was efficiently and thoroughly cauterized immediately after-

TABLE 1

The effect of cauterization upon local skin tuberculosis in guinea pigs produced by avirulent human tubercle bacilli

INTERVAL OF TIME ELAPSING BETWEEN INTRACUTANEOUS INOCULATION OF BACILLI AND CAUTERIZATION	GUINEA PIG SETS	DOSE OF AVIRULENT HUMAN TUBERCLE BACILLI INJECTED INTRACUTANEOUSLY					
		1.0 mgm.		0.1 mgm.		0.001 mgm.	
		Local lesion*	Metastases†	Local lesion	Metastases	Local lesion	Metastases
Controls not cauterized	1	+	±	+	±	−	−
	2	+	±	−	±	−	−
Immediately cauterized	3	−scar	±	−scar	±	−scar	−
	4	−scar	±	−scar	±	−scar	−
One day	5	−scar	+	−scar	+	−scar	±
	6	−scar	±	−scar	±	−scar	±
Two days	7	−scar	±	−scar	−	−scar	±
	8	−scar	±	−scar	±	−scar	±
Four days	9	−scar	+	−scar	−	−scar	−
	10	−scar	±	−scar	±	−scar	−
One week	11	−scar	+	−scar	+	−scar	−
	12	−scar	+	−scar	+	−scar	+
Two weeks	13	−scar	+	−scar	+	−scar	±
	14	−scar	±	−scar	+	−scar	+
One month	15	−scar	+	−scar	+	Not healed	+
	16	−scar	+	−scar	+	Not healed	±

* Local lesion if healed is marked −, if an ulcer remains it is marked, depending upon its size and tendency to heal, either as + or ++, the latter indicating an ulcer about 0.5 cm. or more in diameter and discharging pus with very little tendency to heal, while the former is an ulcer tending to heal and less than 0.5 cm. in diameter.

† The systemic or metastatic tuberculosis in the guinea pigs is graded from − to ++++ as follows: − indicates no visible macroscopic tuberculosis aside from that found at the local lesion; ± indicates a perceptible enlargement of the local superficial inguinal lymph nodes; + indicates a definite enlargement of the inguinal nodes with a slight but perceptible enlargement of the retroperitoneal nodes or spleen; ++ indicates an enlargement of the local inguinal and retroperitoneal nodes and visible macroscopic tuberculosis of the spleen; +++ indicates an enlargement of most of the nodes, definite enlargement and involvement of the spleen with visible tuberculosis in the lungs and the liver; and ++++ indicates a massive generalized tuberculosis of all the organs in the animal.

TABLE 2

The effect of cauterization upon local skin tuberculosis and the metastases therefrom in guinea pigs produced by virulent human tubercle bacilli

INTERVAL OF TIME ELAPSING BETWEEN INTRACUTANEOUS INOCULATION OF BACILLI AND CAUTERIZATION	GUINEA PIG SETS	DOSE OF VIRULENT HUMAN TUBERCLE BACILLI ("GLUCKSON") INJECTED INTRACUTANEOUSLY					
		0.1 mgm.		0.000,01 mgm.		0.000,000,01 mgm.	
		Local lesion	Metastases	Local lesion	Metastases	Local lesion	Metastases
Series 1							
Controls not cauterized	1	++	+++	+	++	+	+
	2	++	+++	+	++	+	+
Immediately cauterized	3	+	+++	−scar	±	−scar	±
	4	−scar	+++	−scar	±	−scar	+
One day	5	++	+++	−scar	±	−scar	++
	6			−scar	+	−scar	+
Two days	7	+	++++	−scar	+	−scar	±
	8	D*		D		−scar	±
Four days	9	++	++++	−scar	+	−scar	++
	10	+	+++	D		D	
One week	11	++	++++	−scar	++	−scar	+++
	12	D		−scar	++	−scar	+++
Two weeks	13	++	++++	−scar	+++	−scar	++
	14	++	++++	D		−scar	+++
One month	15	++	++++	+	+++	+	++
	16	++	+++	−scar	+++	−scar	+++
Series 2							
		1.0 mgm.		0.1 mgm.		0.001 mgm.	
Controls	17	++	+++	++	+++	++	+++
	18	++	++++	++	+++	++	+++
Immediately cauterized	19	+	++	+	++	++	++
	20	++	+++	D		+	++
Two days	21	++	++++	++	+++	+	+++
	22	++	++++	D		++	+++

*D indicates that the animal died before the results were of any value.

ward, and one, two and four days, one and two weeks, and one month after the intracutaneous injection of the bacilli. The local lesion and generalized tuberculosis in the guinea pigs were recorded at postmortem two months after inoculation. The results of this experiment are recorded in table 2.

The experiment recorded in table 2, in which virulent human tubercle bacilli were used for skin inoculation, brings out some important points which were not disclosed by the study recorded in table 1. Cauterization has a beneficial influence upon the local lesion of the skin, even when performed two weeks after the initiation of the lesion and provided the intracutaneous dose of bacilli (0.000,01 mgm. or less) is not excessive; when the dose is as large as 0.1 mgm. the local effect of the cauterization is barely appreciable. When the intracutaneous dose of the virulent human tubercle bacilli is not excessive (not over 0.000,01 mgm.) there is a striking effect of the cauterization of the local skin site upon the general dissemination of the disease, depending upon when cauterization was performed with relation to the infecting inoculation. If the local site of skin inoculation was cauterized within four days after the intracutaneous injection of the virulent human tubercle bacilli, there was present distinctly less macroscopic tuberculosis in the guinea pigs examined two months after infection, while cauterization of the local skin site later than four days after the intracutaneous injection resulted in a distinctly greater amount of macroscopic tuberculosis two months after infection, compared with control guinea pigs only given intracutaneous injections of bacilli without subsequent cauterization of the local site. If the intracutaneous infection was too large (0.001 mgm. or more) the striking differences, both as to local site and general involvement, were to a great extent overshadowed by the massive infection of the guinea pigs.

The above results, in view of Krause's studies on the macroscopic spread of tuberculosis in the guinea pig and the dissemination of bacilli following local subcutaneous injection, become more intelligible. It could hardly be expected that after the bacilli are universally distributed throughout the guinea pig (after about 4 days) that local cauterization could have an appreciable retarding or inhibitory influence upon the resulting metastatic lesions, even though the local effect might be striking. The interesting and surprising point brought out by these studies is, however, that cauterization after this time, even though benefiting the local lesion, causes a distinct increase in the metastatic

involvement. Does this indicate a further seeding internally from the active local site, occasioned by the application of the cautery at this time (immediate embolic or inflammatory embolic), or are we dealing with a toxic injury of sufficient extent to weaken the guinea pig's resistance? The former seems the more plausible view, since, when the intracutaneous dose is small, the results are much more striking in comparison with those seen in animals receiving the larger amounts of bacilli, and this in spite of complete cauterization, which would seem to bespeak immediate embolic dissemination.

DISCUSSION

As has been pointed out by the authors cited above, cauterization is really considered a surgical procedure and, although differing in its mode of application in removing diseased tissue, it may be fairly well compared with the ablation of diseased foci by the knife. There are, however, distinct advantages of the cauterization over the knife, especially when foci produced by pathogenic microörganisms are being considered. The chief and most plausible advantage seems to be the destruction of all living matter, including the pathogenic bacteria, by the cautery, in the field of its action, and thus preventing a direct spread of the infection, as would occur were the operating knife to pass through a contaminated focus. Whether or not cauterization does more than merely cauterize, whether it stimulates cellular resistance and fibrous tissue formation, as claimed by some authors, certainly requires further elucidation and study. It is almost an axiom among surgeons never to operate upon an acute pus focus, such as acutely inflamed tonsils, etc., unless an absolute emergency exists, but rather to wait until the acute stage has subsided. This has been learned by hard and disastrous experience. Likewise, in operations upon scrofulous lymph nodes, when this becomes necessary, the surgeon has learned that it is better to wait for a period of quiescence. Most laryngologists have likewise learned that it is preferable, when treating tuberculous laryngitis by surgical measures, not to attempt interference in the acute fulminating variety, and they are guided by the pulmonary condition of the patient to which the laryngitis is usually secondary and with which it frequently runs parallel. The above cited animal experiments would seem to bear out these conceptions and lend support to the propriety of these methods. If surgical interference is deemed advisable in a localized tuberculosis (tuberculous laryngitis, for example, since

cauterizing has had its strongest advocates in this field) its performance should be carefully guided by the local condition of the disease as well as by the general condition of the patient. If the lesion is quiescent and in abeyance it seems that surgical intervention can be carried out with the least possible danger of dissemination; if, however, the lesion is acute and of the fulminating type it seems that surgical intervention had better be deferred to a subsequent time. The points thus made from these experimental studies should encourage further clinical work, bearing in mind the differences between the lesion suitable and unsuitable for such treatment. They may also have a bearing upon the treatment of skin tuberculosis or localized tuberculosis elsewhere in the body. In the case of the former, especially in certain locations, a distinct contra-indication may exist on account of the cosmetic results due to the application of the cautery. This requires further clinical investigation.

SUMMARY

Cauterization of a local intracutaneous site of inoculation of avirulent human tubercle bacilli (0.001 to 1.0 mgm.) in guinea pigs efficiently destroys and has a beneficial influence upon the local lesion, without tending to affect appreciably the systemic dissemination of the disease, regardless of whether cauterization was performed immediately or one month after the intracutaneous inoculation. When, however, virulent human tubercle bacilli are used for intracutaneous inoculation, cauterization of the local site acts differently. It has a beneficial influence upon the local lesion of the skin, even when performed two weeks after the initiation of the lesion and provided the infecting dose (0.000,01 mgm. or less) is not excessive; when the intracutaneous dose is as large as 0.1 mgm. the local effect of the cauterization is barely appreciable.

When the intracutaneous dose of virulent bacilli is not excessive (not over 0.000,01 mgm.) there is a striking effect of the cauterization of the local skin site upon the general dissemination of the disease, depending upon when cauterization was performed with relation to the infecting inoculation. If the local site is cauterized within four days after infection there is found distinctly less macroscopic tuberculosis in the guinea pigs examined two months after infection, while cauterization of the local site later than four days after infection results in a distinctly greater amount of generalized tuberculosis, as compared with control, untreated guinea pigs given only the intracutaneous inoculation of the bacilli. If the intracutaneous dose of virulent bacilli is large (0.001 mgm.

or more) the striking differences produced by the local cauterization, both in the local site and the general tuberculous involvement, are to a great extent overshadowed by the massive infection of the guinea pigs.

These experiments should have a bearing upon the treatment, by the cautery, of localized tuberculous foci in the human body, especially in selecting the suitable case for this treatment.

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CHRONIC MILIARY PULMONARY TUBERCULOSIS OF LOW VIRULENCE

A CASE REPORT

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The unusual and startling deviation from the ordinary, expected clinical course of miliary tuberculosis is the occasion for this case report. Somewhat similar cases are reported by Bierman (1) and Stivelman and Hennell (2).

S. S., age 59, single, a traveling salesman, was first seen May 20, 1922. His family and past history were unimportant. He gave a history of hawking and spitting over a number of years, with little or no effect on his general health. He had consulted a physician in February, 1922, on account of a moderate sized hemorrhage. His sputum was positive for tubercle bacilli at that time. A few days' rest in bed did away with what slight fever existed and also most of his cough.

Physical examination revealed a robust man, apparently in excellent health and with a slight hacking cough. Temperature, pulse and respiration were normal. His height was 72 inches and weight 173 pounds. The left lung showed a few scattered, wheezy, musical râles, with some questionable dulness over the upper lobe, but no deviation from the normal respiratory murmur and no râles typical of tuberculosis.

The right lung was dull to percussion throughout, with marked narrowing of the isthmus and complemental space. Breath sounds were diminished over the entire right side, with little or no increase in the extent of the bronchovesicular murmur expected normally over the upper portion of the upper lobe. By auscultation could be made out numerous coarse, moist, musical râles over back and front, from apex to base, but more numerous over the lower lobe. Râles could be easily made out without coughing efforts.

Repeated sputum examinations were negative for tubercle bacilli. The Wassermann test was negative. The only complication of importance discovered was a chronic sinusitis, a recent exacerbation of which may have been the etiological factor of his rather typical bronchitis râles, which disappeared a few weeks under bed rest.

An X-ray picture of the chest showed a typical, diffuse miliary tuberculosis of the right lung, without any marked evidences of activity. Several pictures, taken at two or three months' intervals for the next nine months, showed no appreciable differences from the original roentgenograph. No picture was made at the time of the hemorrhage, three months previous to his being examined by me.

A guarded prognosis was given. Much to my surprise, when put to bed in a hospital, the patient ran absolutely no fever, and in a few weeks his cough and expectoration had cleared up. Râles rapidly diminished until, after three or four months, only a few indeterminate râles could be heard at the base of the axilla. Tuberculin treatment was started; O. T., 0.0000001

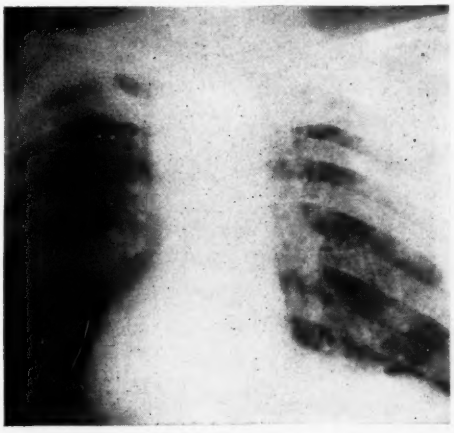

FIG. 1. X-RAY SHOWING DIFFUSE MILIARY TUBERCULOSIS OF RIGHT LUNG

Three serial pictures taken during the following nine months showed no changes from the original picture.

mgm., and gradually in the course of months raised to 0.1 mgm. No reactions occurred during administration. Graduated exercise was started in August, 1922, and on February 1, 1923, the patient was discharged, to resume his usual occupation under observation. His weight at the time of his discharge was 190 pounds. He was examined early in February, 1923, by Dr. J. J. Singer of St. Louis (3), who, by physical examination, could detect absolutely no evidences of tuberculous activity. On March 26, 1923, after a business trip lasting one month, he was examined by me. The examination showed a number of squeaky musical râles over the right back, more marked over the lower lobe. Examination of the sputum revealed tubercle bacilli.

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Christian Saugman
1864-1923

"Our greatest tuberculosis specialist, the renowned Danish professor Christian Saugman, is no more." Thus begins a tribute by Emil Als in the latest number of the *Zeitschrift für Tuberkulose*. Professor Saugman was known to tuberculosis workers throughout the civilized world, and many of the members of the American tuberculosis craft knew him personally, had studied his works, and became his disciples. I believe, therefore, that we on this side of the Atlantic may also bow our heads in sorrow at the passing away of this great and good man.

Saugman was born on December 16, 1864, and died on February 7, 1923. As a student he already showed unusual intellectual abilities combined with deep religious ideality. He had decided to devote himself to surgery, but his contracting a serious tuberculosis, involving the lungs and larynx, caused him to abandon this idea, and shortly after his final examination he went to Goerbersdorf and placed himself under Brehmer's care. He remained in that celebrated sanatorium as a patient and, later on, as an assistant to Brehmer for about two years. He then returned to his homeland and engaged in general practice, but, having become deeply interested in tuberculosis, he determined to establish a sanatorium for the tuberculous in Denmark. He met with a good deal of opposition because of the prevailing idea that the lowlands were unsuitable for the treatment of this disease. In the end he won out, not only with the establishment of a sanatorium at Vejlefjord, but also as leader in a systematic combat against tuberculosis, which resulted in obtaining for Denmark the smallest tuberculosis mortality rate of any country.

Saugman's charming personality was, indeed, a great asset in his work. He was imbued with a sense of duty that prompted him to give every seemingly rational means in the treatment of tuberculosis a thorough trial. With the advent of artificial pneumothorax, he devoted himself to the perfection of the instrument and made the operation safer by the addition of the manometer. His success in the treatment by artificial pneumothorax was considered phenomenal. Although not

trained in surgery, he succeeded in performing Jacobaeus's operation for severing pleuritic adhesions with unusual dexterity. The same may be said of the thoracoplastic operations which made him famous.

Saugman was a born teacher and as such he encouraged and inspired all his pupils who came to him from all over the world to see and to learn the latest in modern phthisiotherapy. The honors bestowed upon him as president of the Danish and Scandinavian tuberculosis associations, the International Artificial Pneumothorax Society, and as honorary member of many scientific bodies were indeed well deserved. His clientele was cosmopolitan; patients came to him from all parts of the globe. The literary contributions by this great Danish phthisiotherapeutist have been numerous during the past quarter of a century and have embraced all phases of the tuberculosis problem. Saugman's labors on behalf of tuberculosis science and the consumptive sufferers will not soon be forgotten by his colleagues and pupils nor by the thousands of men and women now enjoying perfect health and happiness, who bless his memory for what he did for them.

S. A. K.

THE TREATMENT OF SEVERE DIABETES COMPLICATED BY SEVERE TUBERCULOSIS WITH INSULIN[1]

W. D. SANSUM

Santa Barbara, California

The treatment of severe tuberculosis, when complicated by severe diabetes, presents an unusually difficult nutritional problem, because ample nutrition is essential in the treatment of tuberculosis, whereas, in diabetes, the amount of food that may be metabolized is limited by the natural tolerance of the patient. When possible, a diabetic patient must be kept continuously free from sugar and acidosis, in order to prevent a further reduction in his natural sugar tolerance. No nutritional advantage can be gained by feeding the diabetic patient beyond his natural tolerance, because the loss of food in the urine, as sugar and acetone bodies, proportionately lowers his net food consumption.

All forms of infection rapidly lower a diabetic patient's tolerance, and in this combination of diseases we are usually confronted with a rapidly fatal, vicious circle. The infection causes the diabetes to become more severe. As the diabetes becomes more severe, the problem of nutrition becomes more difficult. A stage is soon reached when the patient can no longer be kept sugar-free or free from acidosis and nourished on the most carefully planned diet, even if kept continuously at absolute bed-rest.

It is the purpose of this paper to present the clinical findings and the results obtained by the use of Insulin in three cases. The discovery of Insulin by Banting (1) and his associates of the University of Toronto has entirely changed the nutritional problem in the treatment of diabetes. Patients, when treated with Insulin and regardless of their complications, may be fully nourished even if they have no sugar tolerance. Insulin is not a cure for diabetes and must be administered continuously. We are not ready to promise a cure for their tuberculosis, but we believe that this condition is as curable as it would have been had it not been complicated by severe diabetes.

[1] From the Potter Metabolic Clinic, The Santa Barbara Cottage Hospital, Santa Barbara, California.

CASE REPORTS

Mr. W. S. S., of Manitoba, was first admitted to this Clinic December 8, 1920, with a diagnosis of pulmonary tuberculosis and diabetes mellitus, both extremely severe. He was thirty years of age and weighed 104.2 lbs. The diagnosis of tuberculosis had been made in June, 1920, when the sputum was streaked with blood and contained many acid-fast bacilli. He had been a patient in an excellent tuberculosis sanatorium but because of the existing diabetes he had made no improvement, losing 10 lbs. in six months. On admission to this Clinic the physical findings were those of a generalized involvement of both lobes, with the greatest involvement in the upper right lobe. The sputum contained many acid-fast bacilli. His temperature was continuously subnormal. He remained in the Clinic for approximately three months and gained 10 lbs. on a diet which reached a maximum of 2000 calories, made up of carbohydrate 30.6, protein 38.5 and fat 191.6 grams. He returned to his home in Canada and gained an additional 10 lbs. The following winter his condition became progressively worse. He experienced several attacks of severe bronchitis. He lost the weight he had gained and his diabetes became progressively worse. Finally, in the summer of 1922, he had difficulty in remaining continuously free from sugar on a diet containing 1400 calories. He was readmitted to this Clinic on September 7, 1922, and was the tenth patient in our series to receive Insulin. There was a more generalized involvement of the lungs than before. He was raising large amounts of sputum which contained, as before, many acid-fast bacilli. Previous to January 1, 1923, our supply of Insulin in proportion to the demand was very limited, so that, although we were able to materially increase his diet, we were not able to give him an ample diet. As our supply of Insulin increased, we raised his diet to 3000 calories and on June 15, 1923, his net weight was 158 lbs., a gain of 49 lbs. In the meantime, he had remained continuously free from sugar with moderate increase in his activities. The amount of his sputum had decreased so that it took considerable effort on his part to obtain a specimen for examination. These specimens contained acid-fast bacilli but in fewer numbers.

In addition to the Insulin treatment, he has received ultraviolet light baths from Dr. H. J. Ullmann. We believe this treatment is worth mentioning because we feel that it has had a beneficial effect in his case. For three successive periods, we omitted, experimentally, the ultraviolet light treatment, and in each instance the sputum increased and the patient felt worse, but he promptly became better when the baths were resumed.

Mr. E. J. C. This patient was referred to this Clinic on March 9, 1922, by Dr. Frederick M. Allen of New York, with a diagnosis of severe diabetes

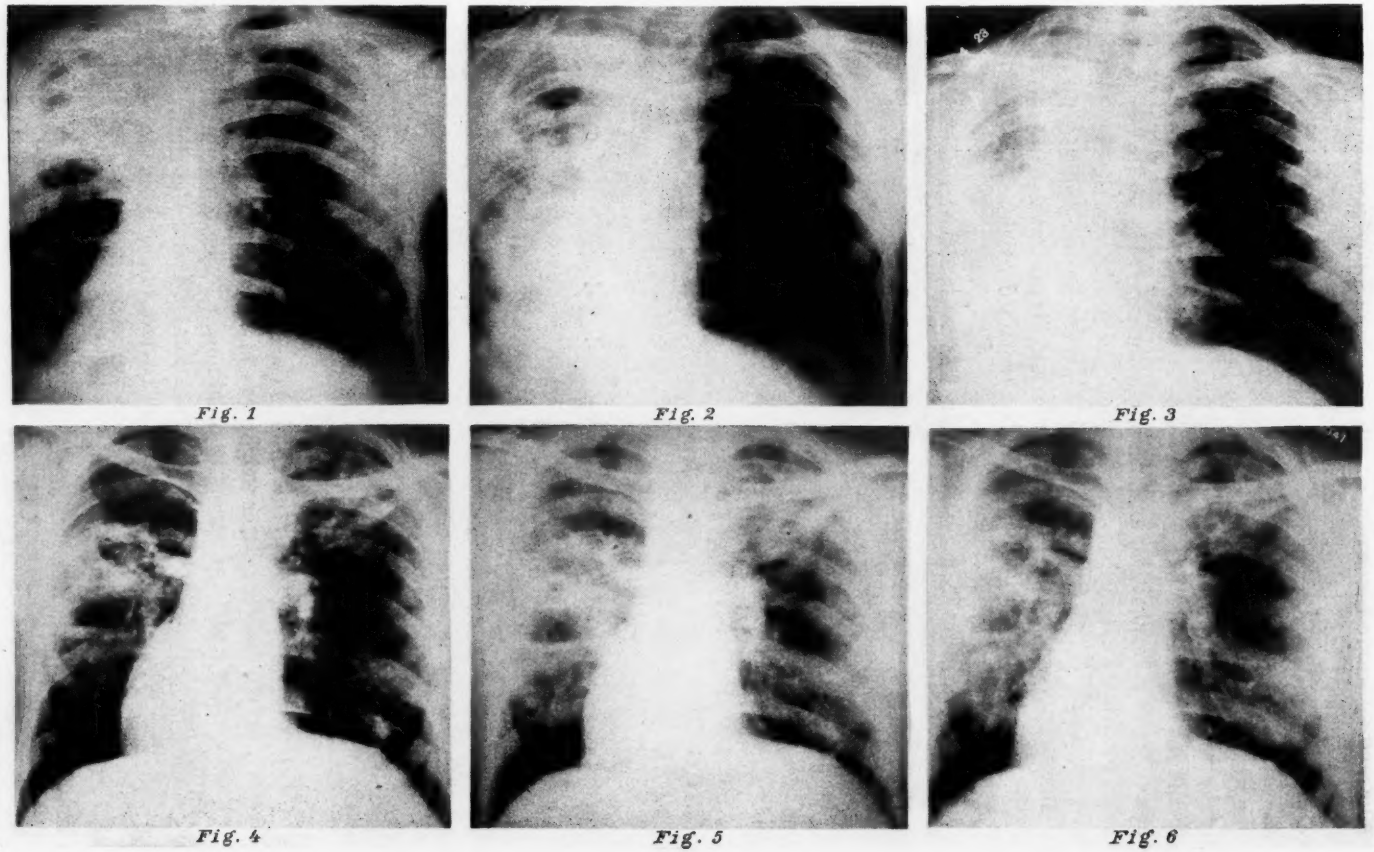

Fig. 1 *Fig. 2* *Fig. 3*

Fig. 4 *Fig. 5* *Fig. 6*

FIGS 1, 2 AND 3. CASE, MR. W. S. S.: JANUARY 8, 1922, DECEMBER 18, 1922, AND MAY 4, 1923, RESPECTIVELY
FIGS. 4, 5 AND 6. CASE, MR. E. J. C., JULY 26, 1922, OCTOBER 23, 1922, AND JUNE 14, 1923, RESPECTIVELY

complicated by severe tuberculosis. His weight on admission was 109.3 lbs. His diet contained 1273 calories, consisting of carbohydrate 9, protein 37 and fat 121 grams. There was a generalized involvement of both lobes of the lung with many acid-fast bacilli in the sputum, which was copious. By keeping the patient almost continuously in bed and by careful attention to his diet, we were able to prevent further loss of weight, but it was impossible to reduce his blood sugar to a normal level. We began using Insulin, in this instance, September 30, 1922; and, although we were able by its use to increase his diet to a moderate degree, our supply of Insulin was then too limited to give an ample diet. On March 10, 1923, when he weighed 116 lbs., we were able to increase his diet to 3000 calories and his present weight is 137 lbs. Aside from an occasional bronchitis, he has remained continuously free from

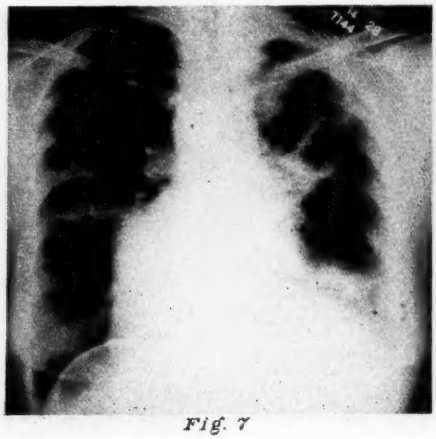

Fig. 7

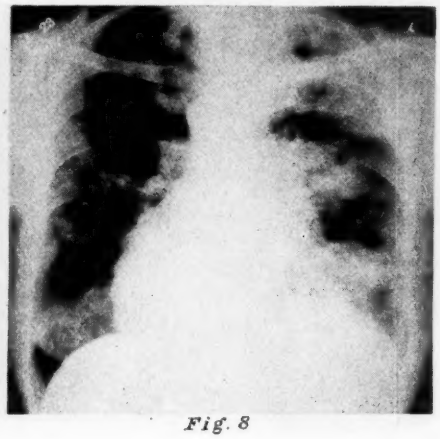

Fig. 8

FIGS. 7 AND 8. CASE, MRS. J. B. F., JANUARY 24 AND JUNE 14, 1923

fever. The sputum has decreased in amount, and while there are still acid-fast bacilli in his sputum they are fewer in number. The patient looks and feels very much better.

Mrs. J. B. F. was admitted to this Clinic January 21, 1923, with a diagnosis of extremely severe diabetes. Her weight was 93.8 lbs. The urine contained 5.7 per cent sugar and large amounts of albumen. Her temperature was continuously elevated, and on routine examination we found the clinical picture of a generalized, extremely advanced and active tuberculosis with cavity formation in the right upper lobe. There were large amounts of sputum which contained many acid-fast bacilli. Centrifugated specimens of urine also contained a few acid-fast bacilli. The patient had been having excellent

diabetic care and could not be kept sugar-free on any diet. Her run-down condition was attributed to her diabetes, which had been present for sixteen years. The patient has been kept on a diet of approximately 2200 calories and was given Insulin on admission, when her natural tolerance was found to be 23 grams of sugar-formers. She continued to have daily elevations of temperature until March 28th, when a series of ultraviolet light baths was given. Following this treatment, and within two or three days of the beginning of this treatment, her temperature became normal and, aside from an occasional elevation, it has remained continuously normal. She has gained a little in weight, now weighing 102.2 lbs. The sputum has materially decreased in amount, but still contains many acid-fast bacilli. We have not been able to feed this patient as liberally as we would have if her appetite

TABLE 1

Summary from food charts of cases

NUMBER	NAME	AGE	DURATION	DATE	NET WEIGHT	DIET	DIET	INSULIN	ILETIN	TOLERANCE	URINE SUGAR	BLOOD SUGAR	ACIDOSIS
			years		*lbs.*	*cal.*	*gms.*	*gms.*	*units*	*gms.*	*per cent*	*per cent*	
10	Mr. S.	30	10	Sept. 5, 1922	102.5	1400	53	0	0	53	0		0
				June 15, 1923	151.0	3069	152	96	113	56	0	0.10	0
14	Mr. C.	33	4	Sept. 30, 1922	115.7	1513	63	0	0	63	0	0.18	0
				June 15, 1923	137.0	2993	163	105	123	58	0		0
62	Mrs. F.	53	16	Jan. 21, 1923	93.8	?	?	0	0	?	0.3		
				Jan. 24, 1923	91.7	1023	62	39	31	23	0		0
				June 15, 1923	102.2	2275	134	117	139	17	0		0

were not so capricious. We do not feel that she has made as much progress as the other two patients reported and we may be dealing with a degree of tuberculosis which will probably result fatally; but, even in the presence of so severe a degree of tuberculosis and diabetes, we have been able to keep her continuously free from sugar and acidosis.

Because diabetes is so frequent and so fatal a complication in tuberculosis, it may be well to sum up the essential points in the treatment of diabetes with Insulin as we understand them to-day. It must be borne in mind that the treatment of diabetes with Insulin is still in its early experimental stage, but since it offers the only hope to such patients as are described above it certainly should be used.

THE ESSENTIAL POINTS IN THE TREATMENT OF DIABETES WITH INSULIN

In the treatment of diabetes, there are three objects to be attained:

1. The patient should be kept continuously free from sugar in the urine and the blood sugar should be normal.

2. The patient should be kept continuously free from acidosis.

3. The patient should be nourished, as evidenced by a satisfactory weight.

These conditions may be fulfilled, in many instances, by careful dietary procedures, although when a patient's tolerance is very low continuous bed-rest is necessary to avoid a serious loss of weight. If the disease becomes progressively worse, as it usually does in severe and untreated cases, a stage is finally reached when the patient can no longer be kept free from sugar and acidosis even if the most careful attention is paid to the diet and the patient is kept continuously in bed.

Many patients wait too long before beginning careful, dietetic management, and specialists in the treatment of this disease are therefore not given a fair chance to do the best work. Dr. Joslin has collected the statistics showing the advantages of careful treatment. Between the years 1814 and 1914, the death rate from diabetes in patients who were treated in the best hospitals was 28 per 100 per year. The year 1914 marked a significant advance in the dietary treatment. The principles outlined in the first paragraph were carefully adhered to. In patients so treated, the death rate has fallen to 4 per 100 per year.

By the use of Insulin, the death rate from diabetes may be reduced to zero, and patients who would otherwise remain chronic invalids may be restored to health by ample diets in proportion as this specific extract becomes available. It is not a cure for diabetes. Patients will need to exercise greater care than ever with their diets, but since these diets will be ample for their needs they will be fully repaid for the additional effort.

In the treatment of diabetes with Insulin, there are four conditions that should be satisfied:

1. The sugar-burning or utilizing power of the Insulin in grams per cubic centimetre should be known.

2. The patient's natural tolerance should be determined in grams of sugar-formers.

3. The exact value of the proposed diet should be known.

4. The dosage of Insulin may then be adjusted to make up the difference between the sugar-formers of the proposed diet and those of the patient's natural tolerance.

The sugar-formers of the diet designated by *G* are 100 per cent of the carbohydrate, 58 per cent of the protein and 10 per cent of the fat. It has long been known that all starches are changed into sugar during the process of digestion. When the protein molecule is digested, it is split into proteoses, peptones and eventually into amino acids. Some of the amino acids may be changed into sugar while others may be changed into fatty bodies. The neutral fats are the glycerolesters of fatty acids. Glycerine is a sugar-former. The reduction of food values and tolerances to the common denominator sugar-formers very materially simplifies the calculations.

1. The sugar-burning or utilizing power of the Insulin should be accurately known. Eli Lilly's *Iletin* is evaluated in rabbit units. Their units have a very constant value since they use between 300 and 400 rabbits in the standardization of each lot. In our experience, each unit is worth a little less than 1 gram of sugar-metabolizing power. Their *H-20* product which indicates 20 rabbit units in each cubic centimetre has a sugar-metabolizing power of 17 grams.

2. The patient's tolerance may be determined by diet alone or by diet plus Insulin. When diet alone is used, the patient is desugarized by partial starvation. When sugar-free, diet additions are gradually made. A diet is eventually found upon which the patient can remain continuously free from sugar in the urine and with a normal blood sugar. Suppose such a diet contains 35 grams of carbohydrate, 38 grams of protein and 83 grams of fat. The sugar-formers of such a diet are 100 per cent of 35 plus 58 per cent of 38 plus 10 per cent of 83, or 35 plus 22.04 plus 8.3, or 65.34 grams. We would say that such a patient has a natural tolerance of 65 grams of sugar-formers.

In the terminal stage of diabetes, patients do not endure starvation well, both because of the existing acidosis and of their already markedly emaciated condition. Many of them cannot be desugarized by any dietary procedure, since their natural tolerances are too low to permit of even bed-rest maintenance diets. In such cases, tolerances may be determined by diet plus Insulin. If the acidosis is not too severe, a patient may be given a bed-rest maintenance diet similar to the above which contains a little more than 1000 calories, with 65 grams of sugar-formers. Small doses of Insulin are given at first. The Insulin is

gradually increased until the patient is continuously free from sugar. Suppose that it requires 60 grams of assistance in the form of Insulin to carry the above diet. The patient's natural tolerance would then be 65 less 60 or 5 grams of sugar-formers.

If the patient has a severe acidosis, no attempt is made to measure the patient until the acidosis is controlled. We control the acidosis by giving the patient a diet rich in carbohydrate, low in protein and as free as possible from fat, such as an oatmeal, skimmed-milk diet, using comparatively large doses of Insulin which, as it metabolizes the carbohydrate, will in turn metabolize the fat and thus dissipate the acidosis.

3. The exact value of the proposed diet should be known. We consider 80 to 100 grams of protein as ample for the needs of an adult. In adjusting the fat, we have followed the formula of Woodyatt's optimal diets, never letting the fat actually oxidized exceed two times the carbohydrate plus one-half of the protein. Following this plan, a diet *G* of 65 will carry approximately a 1000 calory diet and a diet *G* of 120 will carry a 2000 calory diet.

4. The adjustment of the dose: Suppose that the natural tolerance of a patient, as determined either by diet or by diet plus Insulin, is found to be 65. Suppose that the proposed diet contains 133 grams of sugar-formers. The patient will need 133 less 65 or 68 grams of assistance. Sixty-eight divided by 17 will then equal the number of cubic centimetres of Lilly Iletin required, or 4 cc. In our experience, we have found in the majority of patients, with an equal distribution of food between the three meals of the day, that five-eighths of the total dose should be given before breakfast and three-eighths before supper. In this instance, we would give 2.5 cc. before breakfast and 1.5 cc. before supper.

THE SYMPTOMS AND TREATMENT OF OVERDOSAGE WITH INSULIN

When a rabbit is given an excessive dose of Insulin, the blood sugar rapidly falls. When it reaches about 0.04 per cent, convulsions occur which are promptly relieved by the administration of glucose. These convulsions are probably due to osmotic changes incident to the low blood sugar.

Patients may also become too sugar-free from an overdosage with Insulin. This may occur from unfamiliarity with the drug. It occurred with us more frequently in the past, when the strength of the Insulin

was not standardized as well as it is now; especially, as with improved methods of preparation, we were making more potent extracts. It may occur if a patient's tolerance is unknown, and if his diet is not carefully estimated, when it would be impossible to properly adjust the dose, even if the value of the Insulin were accurately known. Overdosage with Insulin excusably occurs in the first weeks of treatment when, by keeping the patient continuously free from acidosis and urinary sugar, his tolerance usually grows very rapidly. Under these conditions, the symptoms of overdosage are usually mild and easily treated if the patient is taught to recognize them early.

THE SYMPTOMS OF OVERDOSAGE

1. *Hunger:* As a patient's blood sugar falls, he usually experiences a keen appetite. This is not a very reliable symptom because nearly all diabetic patients are notoriously hungry.

2. *Slow Mentality:* A patient complains that he cannot think well or concentrate on any one thing.

3. *Extreme Weakness:* This is probably the most reliable early symptom. When a patient is metabolizing an ample net diet, he should feel well and strong; but as he is being overdosed with Insulin a feeling of weakness comes on. Sometimes patients describe this as a dizzy feeling.

4. *Rapid Pulse and Respiration:* The pulse is usually weak and the respiration rapid.

5. *Visual Disturbances:* The patient complains of an inability to read due to double vision or the blurring of the print. He cannot see to write well. The eyes ache and dark spots may appear before them.

6. "*Shaky Feeling:*" The words "shaky" or "the shakes" are terms that have been coined by patients to describe this sensation and, though not particularly scientific, describe the condition very well. The patient simply shakes all over. He is not cold, nor are these symptoms associated with or followed by fever. We believe that this symptom is nature's method of causing comparatively large amounts of glycogen which has been stored in the liver and muscles to be changed into sugar and poured into the blood stream.

7. *Sweating:* The "shaky" feeling is always followed by a profuse sweat. This must be watched for, especially in new patients, since it is a very reliable symptom.

8. *Unconsciousness:* If a patient has been too seriously overdosed, he may become unconscious, falling into a deep sleep from which it is im-

possible to rouse him until sugar has been administered. At this stage, he cannot be forced to swallow fluids.

9. *Convulsions:* If the overdose has been still greater, convulsions may occur. Fortunately, these are very rarely seen in patients, but are a common occurrence in rabbits. The rabbit unit is the amount of Insulin necessary to produce a convulsion in a one kilogram rabbit. In our experimental evaluation of Insulin we have used thousands of rabbits, and in thousands of them such convulsions have been produced; but, despite such marked overdosage, only one or two rabbits have died in the entire series. The intravenous administration of a very small amount of glucose restores such rabbits in two or three minutes.

THE TREATMENT OF THE OVERDOSAGE WITH INSULIN

The treatment of overdosage with Insulin is undoubtedly started by nature as she changes the glycogen stores into sugar and pours them into the blood stream. In this Clinic, whenever a patient experiences the slightest symptoms of being too sugar-free, 20 grams of milk chocolate, having a food value of carbohydrate 10, protein 2, and fat 7, are given at once, followed by or dissolved in hot water. No attempt is made to determine whether the urine or blood is too free from sugar, for we much prefer to give this emergency ration many times when it is not needed rather than to omit it once when it is needed. The milder symptoms, such as hunger, are promptly relieved by serving the tray a little early. The patient in the early stages of treatment is instructed never to leave the hospital without the emergency chocolate ration. Many other substances containing sugar-formers could be used and are used in place of the chocolate. In the beginning we used white crackers, but the starch must be changed into sugar before it can be absorbed. Later, we used whole milk, but half-pints of whole milk do not stay sweet any appreciable length of time and are difficult for the patient to carry around with him. Orange juice, when available, is an excellent form of a rapidly assimilable carbohydrate. Adrenalin may be administered hypodermically which causes the body to rapidly change large quantities of glycogen into sugar. In our experience, the milk chocolate, especially when followed by or dissolved in hot water, has proved to be an excellent form of medication. It is also very acceptable to the diabetic patient who, for so many years, has been without candy. Patients often welcome too sugar-free symptoms because of the chocolate candy reward, and often nibble away at the chocolate to make it last as long as possible when they should eat it as rapidly as possible.

If a patient cannot swallow, glucose should be given by vein at once. We keep on the hospital floors a number of ounce bottles of sterilized 50 per cent glucose and a 25 cc. syringe sterilized for instant use, although we are rarely obliged to use this type of medication. We have had no fatalities from an overdosage with Insulin.

SUMMARY AND CONCLUSIONS

We have described the results obtained in three extremely severe cases of tuberculosis complicated by extremely severe degrees of diabetes mellitus, showing that by the use of Insulin the nutritional problem can be adequately cared for regardless of how grave the diabetes may be.

We believe that by the use of Insulin a patient will have the same chance of recovery as he would have had if his tuberculosis had not been complicated by diabetes.

REFERENCE

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A CASE OF DIABETES COMPLICATING CHRONIC PULMONARY TUBERCULOSIS TREATED WITH INSULIN

(A Preliminary Report)

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The man who forms the subject of this report broke down with pulmonary tuberculosis in 1919, following what was diagnosed as acute influenza. He was a large man, who had always had excellent general health; his family history was negative except for one brother having diabetes; his past history was unimportant except for gonorrhoea some years earlier.

In the spring of 1919, after an acute illness, called influenza, he did not regain his strength or energy. In the summer of the same year one physician found sugar in his urine and told him that he had diabetes; shortly afterward another physician examined his urine and found no sugar in it. In the autumn of 1919 a diagnosis of pulmonary tuberculosis and diabetes mellitus was made by the Battle Creek Sanitarium. His blood sugar at that time was 0.19 and the urine sugar-free. He came to our institution in November, 1919, for treatment for tuberculosis; his urine examined on a number of different occasions was found to be free from sugar.

As he did not make satisfactory progress on the ordinary rest cure, he was given artificial pneumothorax. Within a few weeks he developed tuberculous hydropneumothorax, which quieted down in a short time, so that he was able to leave the sanatorium. His progress was satisfactory and he was able to be up and about until October, 1920. At that time, following a severe cold, he developed empyema. Drainage by means of a pneumothorax needle and lavage was used, and his condition improved; his temperature approached normal until January, 1921, when he had a flare-up of temperature and symptoms. At this time sugar reappeared in his urine; it quickly disappeared upon restriction of diet. Treatment of the empyema with gentian violet was begun at this time; in ten weeks he was free from fever and the fluid in his chest was serous rather than purulent. Contrary to advice, he began to take exercise and eat any food he liked; during the late spring of 1921 he had a recurrence of the empyema and also of his diabetic symptoms. Gentian violet treatment was resumed at once, together with a rigid restriction of diet; in three months he was again free from sugar and pus in his pleura. Since that time, there has been no recurrence of his empyema in spite of the fact that his diabetes has progressed.

The present condition of his chest is as follows: The right lung which had been collapsed is about half reëxpanded; there is no fluid in the pleural space on that side; on the left there are three small cavities at the level of the second rib; otherwise the left lung is clear; the sputum is purulent and contains tubercle bacilli; he rarely runs fever at present.

During the winter of 1923 his diabetes became worse; sugar did not disappear from his urine so quickly after restricted diet, and he rapidly lost weight and strength. On March 17 he entered the Sanatorium again for treatment with Insulin.

At this time his blood sugar was 0.333; the 24-hour quantity of urine was 2680 cc., specific gravity 1.030, color amber, reaction acid, albumen none, acetone and diacetic acid both present, no casts, pus, or blood. The sugar excreted during the twenty-four hours was 13.875 grams. He was observed, without altering his diet which was rather lax, until March 19th, when his blood sugar was 0.4 and he began to be drowsy and show symptoms of approaching coma.

Insulin was begun in very small doses,—2½ units before each meal, and cautiously increased. His diet was set at 50 grams protein, 50 grams carbohydrate, and 130 grams of fat; the object was to furnish him with at least 1300 calories daily.

On March 20th, a fasting blood sugar estimation was made. It was 0.333. Ten units of Insulin were given, and two hours afterward another blood sugar estimation gave a figure of 0.25. At 5 P.M. of the same day his urine was free from acetone and diacetic acid, and in the 24-hour quantity there were only 3.8 grams of sugar.

On March 21st, at 11 A.M., his blood sugar was 0.266. On that day he received, in all, 35 units of Insulin, given in 3 doses before meals.

On March 22nd, his blood sugar at 11 A.M. was 0.22. During the day he received 40 units of Insulin. That night the patient thought that he had a reaction to the drug, and complained of feeling dizzy and "out of his head." We doubted whether this was a true reaction, as it occurred four or five hours after his last dose of Insulin and also about four hours after his supper.

On March 23rd, the blood sugar at 11 A.M. was 0.255; at 5 P.M. it was 0.25; and on this day he also received 40 units of Insulin.

On March 24th, the blood sugar at 11 A.M. was 0.25; on this day he was given 45 units of Insulin.

On March 25th no blood sugar estimation was made, but he received 37½ units of Insulin.

On March 26th at 5 P.M. the blood sugar was 0.111; the urine was entirely sugar-free; the urine examination was negative in all respects. On this day he was given 37½ units.

On March 27th, the blood sugar was 0.1. On this day it was planned to start increasing his carbohydrate intake, but the patient again left our care against medical advice, hoping to be able to continue the Insulin in his own home.

In all, it required 278½ units of Insulin to bring his blood sugar down from 0.4 to 0.1. This amount was administered within nine days, without any untoward symptoms of any kind. The Insulin used was furnished by the Eli Lilly Company and was found to be potent in every respect.

SUMMARY

It is of interest to know that it has been possible by the use of Insulin, controlled by blood sugar estimations, to handle a severe case of diabetes complicating chronic, active pulmonary tuberculosis, and bring the blood sugar down within nine days from 0.4 to 0.1 and rescue the patient from the brink of diabetic coma.

SOME OF THE MORE FUNDAMENTAL REACTIONS OF THE BODY CELLS IN TUBERCULOSIS[1]

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Tuberculosis is defined in modern textbooks as being "a general or local infectious inflammatory disease, the result of the implantation and proliferation of the tubercle bacillus." This definition, while broad enough to include all effects produced by the bacillus, usually conveys a dominant morphological conception. This is largely because the morphological conception is most emphasized in our present state of medical advancement. It fails, however, to emphasize sufficiently the all important fact that the full disease picture represents not only the anatomic changes, but also the functional disturbances which take place in both cells and organs in a human being possessed of a physical body and a psychic system, both of which are influenced and controlled by, and influence and control, in turn, nervous and endocrine systems. It is worthy of mention that, in his contribution on tuberculosis in *Nelson's Loose Leaf Medicine* (vol. i, p. 309), Krause defines tuberculosis as "any pathological formation set up in the animal body by the presence of . . . . the tubercle bacillus, as well as any functional disturbance that results from this anatomic change or the activities of the bacilli."

THE PROBLEM STATED

If ever we are to understand why one patient breaks down with active clinical tuberculosis, while another with the same infection does not; or, if we are to understand why one lesion will heal, while another one with apparently the same extent of infection will not; in short, if we are to understand the principles which underlie the defense of the body against the bacillus, we must not be satisfied with the usual conception of tuberculosis, but we must understand what cellular activity means; what causes it; what interferes with it, and what part it takes in the bodily reaction to disease. Such a study leads us to an attempt

[1] Read before the Philadelphia Clinical Society, November 7, 1922.

at understanding the physical cell; its properties and functions; its reactions; its relationships to other cells; the chemical and nervous reactions which influence it and are influenced in turn by it, and the manner in which these influences originate in and affect both the physical and psychical systems of man. We cannot travel far in this direction at this time because the course has not yet been established, but it is our duty to examine and familiarize ourselves with the reports of those who have made the early surveys, that we may help in the construction when sufficient material is at hand.

All life is phasic or oscillatory in character. There is a period of action followed by a period of inaction. The inaction is the period of rest, during which time energy for action is acquired or regained. This applies to each cell, as well as to all tissues and organs of the body. The processes which characterize life, as compared with death or inactivity, go on within the cell itself, but are influenced by outside stimuli. They are connected with metabolism, with oxidation and the formation and discharge of CO_2.

Anatomic, pathological and bacteriological studies have lifted the science of medicine to-day to a much higher plane than that on which it rested before these special lines of investigation were brought to their present degree of development, but they still leave much to be desired. At present progress is being hindered because we do not possess sufficient knowledge of the fundamental reactions of the cells that make up the body. The normal reactions and the control of the body cells, under conditions of health and disease, stands to-day as one of the most inviting fields for research. It is necessary for us to understand the inherent activities of the cell and the manner in which these are influenced by nerve stimulation and chemical reaction. It is to physiology, both normal and pathological, and biochemistry that we must turn for the truths which will lead us beyond our present knowledge.

In order to understand our problem fully we must realize that salutary, as well as harmful, stimuli may arise in either the physical or the psychical system and disturb the other; or in either system and produce further disturbance in that system. It must be further understood that altered function and the changes in physiological activities alter the anatomic condition of the cell itself, and that alteration in the anatomic conditions of the cell, on the other hand, changes physiological activity. Therefore, a disturbance, either of an anatomic or of a physiological nature, may start a vicious circle which will involve both form and

function before its action ceases. Not only is it necessary for us to have both an anatomic and physiological conception of the human machine, and recognize the psychical part of man, but we must go deeper than this and have a definite conception of the innermost workings of the cells which make up the human structure.

THE CELL AND CELLULAR ACTIVITY

The physical machine is composed of innumerable cells. It is the conception of the biochemist that the basis of these cells is colloidal in character. These colloidal systems differ in different organs and tissues, and in the same organ under different circumstances. They hold in labile form crystalloidal substances, which they either bind more firmly or give off during normal activity or when their equilibrium is disturbed from without. These cells are stored with potential energy. Moore (1) states, "they have the power to start energy, to stop it when started, to bring energy from without and to transform it into utilizable energy, and to link activities for the purpose of producing new reactions."

Biochemists and physicists recognize that there is quite a possibility that the activity which takes place in cells is at least partly of an electrical nature. This theory was early supported by Nernst (2) (3).

Moore (1) discusses this point as follows:

> As to the mechanism or type of energy transformation by which the cell does its work nothing is known; similar phenomena of concentration of ions and of dissolved colloids by means of movement in the electric field have long been known and it is probable that it may be the case that the living secreting cell, by developing differences in electrical potential at its two ends, or by developing differences in energy potential of some other form of energy such as that which intrinsically belongs to the living cell, may establish a directive influence upon substances in solution as a result of which, and of energy potentials upon the dissolved molecules themselves, they may be caused to move in a definite direction and at a definite speed through the cell, different from that of the water in which they are dissolved. A similar directive movement, in fact, to that seen in the case of dissolved ions and colloidal molecules in the electric field may occur.

Crile (4) has recently advanced a theory of life and action based upon cellular activity being of an electrical nature, and in this theory he explains not only many of the changes that take place physiologically, but also pathological influences which are exerted upon the human

being by harmful stimuli and disease, and the process which we recognize as death.

Under varying physiological conditions the colloidal systems of the cells themselves may change. They may either bind more firmly or give off water and various crystalloidal substances which they hold. Pathological conditions are alterations, either diminutions or exaggerations of these normal physiological actions. Anatomic change may be responsible for a disturbed function and the reverse is no less true. Body cells live in water and exist in a very narrow acid-base equilibrium. This normal acid-base equilibrium of the tissues depends upon the colloids binding or giving off electrolytes in certain proportions.

The factors in our colloido-crystalloidal conception which seem to be disturbed by disease processes are as follows: First, the phases of the colloidal systems which make up the normal tissues may be changed; second, the ability of the colloids to bind or give off electrolytes may be interfered with; third, the electrolytes which combine with the colloidal systems may not be present in normal amounts; fourth, the water content of the body in which the colloids of the cells exist and which furnishes the medium for carrying the electrolytes to and from the cells, and in which the cells work, may be insufficient; and fifth, differences in surface tension may exist.

The normal acid-alkali balance, and the manner in which it is disturbed by disease processes, has received considerable attention in recent literature.

The foundation for this knowledge was laid by Walter (5) about fifty years ago and the early clinical observations were made by Stadelmann (6) in his study of diabetic coma. Other observations on diabetes and nephritis were made from time to time but the foundation for the intensive study of this condition is found in the work of Henderson (7), who published his epoch-making studies in 1909. These were followed by those of Benedict (8), Rowntree (9), Van Slyke (10) (11) (12), Sellards (13) (14) and many others, who have shown that the acid-base equilibrium of the body and its variation in disease is one of the most important studies connected with the reactions of the human body to disease. The normal hydrogen ion concentration varies between 7.3 and 7.5 and the greatest range compatible with life is 6.95 to 7.8.

It is necessary that the normal equilibrium between acids and bases in the tissues be maintained. In order to do this the excessive acid radicals which arise during metabolism must be gotten rid of. The

principal ones are those arising from the oxidation of sulphur, phosphorus and carbon. These are found in such great excess over the bases of the body that they would prove disastrous to cellular life if not eliminated.

(1) Carbon is readily oxidized and eliminated as CO_2 by the lungs and by the kidneys after being converted into urea.

(2) Some of the acids arising from sulphur and phosphorus are neutralized by the fixed bases of the body. The kidney will also eliminate acids as acid phosphates and acid carbonates.

(3) In the metabolism of nitrogen the acid radicals are neutralized by ammonia. While the maintenance of the proper acid-base balance is easy of accomplishment in health, it is quite different in disease.

An increase in acid radicals occurs in certain diseases and may ultimately result in a depletion of the body bases.

It has been taught by many students (Fischer (15)) that the water content of the tissues is increased by conditions of increased acidity or alkalinity, and that a normal amount of free water is necessary to physiological action (Balcar, Sansum and Woodyatt (16), Schmidt and Sansum (17)). So anything that will alter markedly the acid-alkali balance of the body will cause the colloids to take up or give off more water. Toxic states, among others, produce this effect, as will appear later. The effect of a disturbance in the normal power of the body cells for binding water is important and far-reaching in its effect upon the physiological organism. If more than the normal amount of water is bound to the colloids, the free water of the body is diminished, a condition which we have recently learned to recognize and avoid when possible in clinical practice.

It readily can be seen how such altered conditions of the cells might interfere with their function of producing specific enzymes which are necessary to defend the body against harmful agencies such as bacteria.

When Sydney Ringer (18) (19) (20) perfected the solution which bears his name, he established the important relationship between the calcium and potassium ions in the cell and pointed the way which has been all too tardily followed for understanding the nature of cellular action. Sodium chloride solution had been used for the histological examination of fresh tissues and for the dilution of blood and had proved satisfactory, but Ringer found that muscular contraction of the heart of the frog could not be carried on long when a saline solution, isotonic with the blood, was used for perfusion. He then added calcium

and the heart began to contract again, but diastole was prolonged and imperfect. He then found that by the addition of potassium diastole was strengthened and the heart beat with forcible systole and diastole. He further found that a slight amount of sodium bicarbonate, when added, improved the contraction very markedly. Ringer recognized that the value of the sodium was to render the solution alkaline like the blood itself. While these observations were made back in 1881 to 1883, it is only now that medicine is beginning to appreciate their clinical application.

The energy of the cell is manifested largely by its binding and setting free these ions. In the heart experiment of Ringer, as we interpret it today, we note that calcium, in controlling the systole, acts the same as stimulation of the sympathetic nerve going to the heart, and that potassium, in controlling the diastole, acts the same as stimulation of the vagus. Thus we conceive of cellular action being a process in which the cell takes up oxgyen and forms chemical compounds with it and the ions which it (the cell) already holds. This causes a shifting of the ions held by the cell. In action the cell again changes its content of ions and awaits the new supply of oxygen. In this way the cell charges and discharges, and so manifests an innate power to do work. There is a normal rhythm which is possessed by the body cells in which action is followed by rest. This rhythm is due to or at least accompanied by changes in the colloids themselves and in their content in *ions*.

EFFECT OF NERVE STIMULATION ON CELLS

The effect of nerve stimulation in this biological conception is a very interesting one. When a nerve is stimulated and the impulse carried to the body cells, its effect is that of making the colloids change their content in electrolytes. Howell (21) found that the inhibitory action of the vagus upon the heart was absent without the presence of potassium, and Howell and Duke (22) found an increase in the potassium content of Ringer-Locke's solution which had passed repeatedly through the heart of a mammal under vagus stimulation. From this, Howell called attention (1906) to the fact that vagus action was in some way connected with the setting free of potassium ions. This has been confirmed recently by Zondek (23), Abderhalden (24) and others. This work shows that stimulation of the sympathetic nerve causes the heart to throw out into the blood stream certain electrolytes, notably calcium,

which, when perfused through another heart, causes the same action as though the sympathetic nerve going to that heart were stimulated. Stimulation of the vagus nerve, on the other hand, causes the colloids of the heart to throw out potassuim ions, which, when perfused through another heart, causes the same action as though its vagus were stimulated. This same ionic action is taken into account in the intestinal tract. Sodium and potassium ions stimulate the intestinal tract, the latter, particularly, producing the same effect as though the vagus were stimulated, while calcium relaxes the intestinal muscle the same as though the sympathetic were stimulated. Nerve stimulation under this conception produces an alteration in the colloidal content of electrolytes, and the suggestive fact noted by the above observers is that the sympathetic and the vagus affect different electrolytes. It is further important to know that the result of a given nerve stimulus may differ according to the relative amounts of calcium and potassium present in the cells and according to their concentration. In fact, under certain conditions of varying amounts of calcium and potassium, as well as in conditions of increased excitability of the sympathetic and parasympathetic nerves, even reverse nerve action may be produced. From these experiments we see that calcium in the cell is closely related to sympathetic stimulation, while potassium is related to vagus stimulation.

Calcium has been used for its relaxing effect on the musculature of the bowel in intestinal tuberculosis. Its effect is to oppose the action of the vagus which contracts the musculature causing spasm; hence its administration relieves pain. The writer has recently used it in some severe cases of asthma with gratifying results, showing that here too it has the same effect as stimulating the sympathetic nerve, which antagonizes the vagus and causes the bronchial spasm to relax.

Important discussions of the ion effects upon the cells may be found in recent articles by Sakai (25), Daly and Clark (26), Kolm and Pick (27), Andrus and Carter (28), Hammett (29), and a splendid summary by Sollmann (30). Such studies should receive careful attention of clinicians because they aid in understanding the manner in which the body cells react in health and disease.

PROTEIN DIGESTION AND TUBERCULOUS TOXEMIA

We must now inquire how pulmonary tuberculosis affects cellular activity. Most of the harmful influences exerted on the body by pul-

monary tuberculosis are of a toxic nature. The local effects of the disease on the pulmonary tissues and the reflex disturbances in other tissues, while annoying, are rarely serious.

The source of the toxemia is probably a complex one. Much of the discussion on this point has assumed that it was of bacillary origin; but it is now evident that the splitting of native protein (pulmonary tissue) is an important source of toxic materials (31). We recognize this as an important source, now that we know that native protein in its course of degradation contains the same elements of toxicity as foreign protein (Vaughan (32), Shittenhelm, Weichardt and Grisshammer (33) and Opie (34)). Krause (35) has presented cogent reasons, some of them deduced from animal experimentation, in support of his belief that much of the intoxication of tuberculosis is due to nonspecific products, derived from the cellular constituents of tuberculous processes.

Any protein which is digested outside of the alimentary canal, and outside of the body cells themselves, is likely to produce toxic symptoms, because the intermediate products of protein digestion, when found parenterally, are toxic.

Protein digestion is carried on by certain enzymes. These are found in the tissues and become active in the presence of disease. Some of these break protein down into its toxic states and leave it to circulate in that condition. Others carry the digestion through to the amido state, producing complete detoxication.

Petersen (36), in discussing the extracellular degradation of protein, calls attention to the fact that the dead leucocyte produces three distinct enzymes which carry on this function: (1) one which acts in a slightly alkaline medium, which has the power of splitting protein mainly to the toxic stage; (2) a more active one which acts when the acid-alkali base is shifted toward the acid side and having the same effect on protein; and (3) an erepsin-like enzyme which acts in a neutral medium and has the power of hydrolyzing protein from the toxic intermediary states such as albumoses and peptones to the nonpoisonous amino-acid states. Thus, enzymes (1) and (2) are responsible for the production of toxic substances, while (3) has a detoxicating influence, but unfortunately cannot act in an acid medium.

The breaking down of protein gives rise to sulphuric and phosphoric acids which are large factors in causing the normal acidity of human urine. During disease there are many factors which come in, not only to increase this acidity of the urine, but to shift the entire acid-base

equilibrium of the tissues toward the acid side. The destruction of bacteria, native protein and toxins furnishes extra sources of acids; so does incomplete oxidation, and a restricted diet or a diet poor in carbohydrates. This increased acidity of the body tissues, according to Petersen, causes a binding of antiferment and frees the proteolytic enzymes, so that they can carry on their digestive processes. At the same time it provides the condition favorable for the working of that particular enzyme which acts in a reduced alkaline medium and which is most active in splitting protein into toxic molecules; and, inasmuch as the erepsin-like enzyme requires an alkaline state for its activity, it cannot detoxicate the peptones and albumoses which have been produced. Thus, the mild acidosis accompanying tuberculous toxemia is partly produced by the breaking down of protein, and at the same time this acidosis produces conditions favorable for a continuance of breaking down of the protein molecules into toxic substances.

Another source of increased intoxication in the tuberculous patient is exercise when the disease is active. This may act in two ways. If the body is in such a condition from the state of the disease that it cannot carry on oxidation completely, this will result in retention of an increase of acid ions, the effect of which is evident from the preceding discussion. Again, exercise hastens circulation and forces an increased number of toxic products into the circulation, whose parenteral digestion further increases the acid balance and exerts a general harmful influence.

Further harmful influences resulting from the shifting of the acid-alkali balance are exerted on the colloids of the body cells. They are made to bind more water than they do under normal conditions, which of itself has harmful consequences for the cells. They are also caused to alter their content in electrolytes. The presence of acids in excessive amounts exaggerates the sympathetic stimulation (30) which disturbs metabolism and causes among other effects inhibition of action throughout the gastrointestinal tract.

THE TOXEMIA OF TUBERCULOSIS AND THE NERVOUS SYSTEM

We must assume that the cells of the nervous system are influenced in like manner as other cells, differing only according to the nature of their peculiar chemical structure. This, together with the changes in the colloidal arrangements of the cells which they act upon, and the

cells' altered binding power for water and electrolytes, must suggest ways in which toxins exert their harmful influences. Toxemia is accompanied by a condition which is expressive of widespread nerve instability, affecting all functions of the body more or less. In those symptoms which arise in the visceral system, however, the expression in states of acute toxemia is predominantly that of sympathetic stimulation, as is evident on carefully analyzing the symptoms.

The effect of toxemia is to interfere with many of the important functions of the body and, if long continued, to cause general nerve instability and permanent injury to physical processes.

A depletion of the cells in their sodium, calcium and potassium bases accompanies chronic toxic tuberculous states, and from our knowledge of the relationship of the cell content in these bases to action and inhibition of action of the sympathetic and parasympathetic components of the vegetative system, the marked nerve instability which is sometimes manifested in acute and chronic toxemia is at least partially explained.

In attempting to locate the action which results in nerve instability, fatigability and nerve exhaustion in some particular portion of the neuron, it has been suggested by different observers that the harmful influence may be exerted on either the central nerve cell, the synapse or at the neuromuscular junction.

Fatigue products are the normal accompaniments of cell activity. If they are present in small quantities they stimulate the cells and result in their own expulsion. If these products accumulate in larger quantities, however, they have a retarding or benumbing influence upon the cell, which slows its activity and interferes with the expulsion of waste products.

Investigation has shown that fatigue causes an increase of hydrogen ion concentration at the synapse.

While we may not be able to analyze and correlate the many results of various workers, and present the facts in such a way as to convince ourselves that we are now able to fully explain the action of toxins in disease, yet we must look upon the knowledge which we possess as being suggestive and partially satisfying. Recent investigations hopefully point the way and show in what fields of investigation future progress is most likely to be made.

With present knowledge we are now able to define pulmonary tuberculosis as a general or local infectious inflammatory disease, usually

chronic in nature, resulting from the implantation and proliferation of the tubercle bacillus and producing its ultimate changes in the body cells, altering their physiological activity and disturbing both the physical and psychical reactions of the individual.

TOXIC SYMPTOMS OF TUBERCULOSIS

Now let us discuss more particularly the "toxic" symptoms of tuberculosis. Their clinical expression is what we have been accustomed to consider as indicating harmful nerve stimulation. We meet the same group of symptoms in acute and chronic toxemia, differing, of course, in degree and in the individual symptoms present in any given case. We meet the same in acute and chronic fatigue, either of physical or mental nature; in conditions of disturbed nerve equilibrium, whether of physical or psychic origin; in many of the conditions which we have learned to designate as endocrine disturbances; and see them following such conditions as overwork, insomnia, dissipation and worry (Pottenger (37)).

These symptoms are those which we usually ascribe to acutely or chronically disturbed nerve equilibrium. Some of them, such as malaise, loss of strength and nerve instability, represent a general harmful nerve action, while others, such as rapid heart, loss of appetite, digestive disturbances, night sweats and rise of temperature (partly only), may be more particularly assigned to action through the sympathetic division of the vegetative system. It is also evident that during states of toxemia the chemical control of body structures, as found in the products from the glands of internal secretion, and the action of the body cells which depends upon the state of the colloids and their content in acids and bases, likewise have a share in producing disturbances in general physiological action; for the vegetative nerves, the endocrine glands and the electrolytic content of the cell colloids are all combined in the control of body action.

It readily can be seen from the nature and the wide distribution of the toxic symptoms that they are the serious symptoms of this disease. They are those which are most likely to prove serious, and the ones that are most difficult to control with therapy.

Rest is the most generally applicable and the most efficient of all remedies with which to combat toxic symptoms. It saves the patient from much of the shifting of the acid-base equilibrium toward the acid side

which follows exercise, and it lessens the circulation about the tubercle which washes the toxic substances into the blood stream. Hence it aids in preserving the acid-base equilibrium in a state as nearly normal as is consistent with the disease present.

Alkalization is of great value in supplying the bases which are withdrawn from the tissues as a result of the increased acid production; in reducing the pathological acid content of cellular colloids and allowing them to give up water which they are pathologically binding; and in restoring the natural state of alkalinity which is necessary to the normal activity of the body cells and in which protein is most easily split into the amido acid state, thus avoiding the intermediate toxic states. Alkalies should be accompanied by large amounts of water.

Artificial pneumothorax aids, and is indicated in a certain small group in which the toxic state and the progress of the disease cannot be controlled by other measures, provided the lesion is largely one-sided; and, if the other lung is not involved, the lesion is wholly or largely quiescent.

Carbohydrate foods should be used as freely as possible and should make up a large part of the dietary, for proteins produce large quantities of acid radicals, which must be satisfied by the body bases before they can be rendered innocuous or excreted.

The most important conclusion to be drawn from our knowledge of the effects of toxins is the tremendous importance of sparing the patient their serious consequences. The difference in prognosis between the early clinical tuberculosis before the toxic state has been reached and the advanced condition accompanied by the toxic symptoms, can well be compared with a slightly inflamed appendix and one that has ruptured. Early diagnosis and early intelligent treatment will prevent nearly all advanced toxic cases of tuberculosis.

THE REFLEX SYMPTOMS OF PULMONARY TUBERCULOSIS

The reflexes which arise in the inflamed area in pulmonary tuberculosis are of immense interest to the clinician. Although they are not as serious as the symptoms which are produced by toxins, yet they are at times exceedingly annoying. They affect many widespread tissues of the body and, if one only understands the nerve paths through which these reflex disturbances may occur, his conception of the many little annoying things that arise in the course of active tuberculosis will be more intelligent.

The lung, being supplied by filaments from both the sympathetic and the vagus of the parasympathetic division of the vegetative nervous system, may produce reflexes in many and widely distributed tissues of the body.

The sympathetic afferent fibres go back from the lung to the upper five or six thoracic segments of the cord. From here we find that the impulses are conducted by intracentral paths upward in the cord to the cervical segments, where they are transferred to cervical motor nerves with which they complete muscular reflexes, and to cervical sensory nerves, through which they produce those sensory disturbances in the skin, subcutaneous tissue, muscles and other deep skeletal structures which accompany pulmonary tuberculosis. These same nerves furnish the paths through which the trophic reflexes are produced.

There are also visceral reflexes produced in those tissues which receive sympathetic fibres from these upper thoracic segments, too, in which both the afferent and efferent paths belong to the sympathetic system. One reflex of this nature is the disturbance in equilibrium, usually a dilation, of the pupil on the side of involvement.

The vagus carries stimuli to the medulla which are here transferred to other branches of the vagus and to all other nerves which are in reflex relationship with it. In this way we offer a rational explanation for the discomfort noted in the area supplied by the sensory fibres of the 5th and the motor reflexes noted in the tissues supplied by the 5th, 7th, 9th, 11th and 12th cranial nerves.

The most important of the vagus reflexes are those which express themselves in other branches of the vagus nerve; those in the larynx, through both the superior and inferior laryngeal nerve; those in the pharynx, esophagus, stomach and intestines; those in the heart; and those in the lung itself, through the respective branches of the vagus.

In considering pulmonary reflexes, the developmental origin of the lung from the gastrointestinal tract must not be forgotten, for it is in all probability due to this fact that reflexes from it so often disturb the smooth running of the digestive system.

In the foregoing discussion I have endeavored to apply in the clinic some of the more important facts which have been brought out in recent years in the physiological and biochemical laboratories, and show the important bearing which they have upon the understanding of clinical tuberculosis.

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THE STANDARDIZATION OF TUBERCULIN

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In the course of studies on progressive tuberculous infection of guinea pigs we have sought to apply the intracutaneous tuberculin reaction, the essential facts with regard to which were developed by Roemer (13). Much of our work has been done with various extractive preparations other than old tuberculin. Incidentally, however, we became impressed with the belief that this method might afford the basis for an improvement in the current procedure of tuberculin standardization. We have therefore conducted and here present one experiment with the view of emphasizing this possibility.

The present method of determining the potency of tuberculin has been developed on the basis of the observations of Koch (1) (2) that tuberculous guinea pigs and normal guinea pigs react differently to injections of tubercle bacilli, that the sensitiveness of tuberculous animals to tuberculin increases as the tuberculous process advances, and that, as the disease advances they succumb to a dose of tuberculin which is insufficient to kill animals suffering from a less extensive tuberculosis. Autopsy of animals killed by tuberculin revealed characteristic pathological changes such as congestion and edema at the site of injection as well as congestion of adjacent lymph nodes. The spleen and liver showed congested patches varying in size, while in the lungs the same changes occurred, but to a less marked degree. The small intestines were found to be markedly congested. On the basis of the foregoing observations, Koch considered a tuberculin sufficiently potent for general use if 0.5 cc. or less would kill, in six or thirty hours, a guinea pig infected three to four weeks previously with tuberculous material, provided that at autopsy the characteristic pathological changes were found.

Donitz (3) improved upon the method of Koch by infecting his animals with the same quantity of a nine to eleven day old culture of tubercle bacilli. When the animals showed some loss of weight, about the second or third week, several were injected with a standard tuberculin and

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their sensitiveness to tuberculin determined. The animals were then divided into two sets. One set received the standard tuberculin in varying quantities, while the other received varying quantities of the tuberculin, the potency of which was to be determined. Donitz considered the minimal lethal dose that amount of tuberculin which would kill a tuberculous guinea pig, of average sensitiveness, in twenty-four hours. A comparison of the lethal dose of the standard tuberculin with that of the unknown tuberculin was an index of its potency. This method, with some modifications, is the one employed by the Institute for Experimental Therapy, Frankfort a. M. (4).

In the United States the function of testing the potency and purity of tuberculin devolves upon the Bureau of Animal Industry which employs the following method for determining the potency of tuberculin (5): A carefully measured minute quantity of tuberculous material, obtained from a tuberculous guinea pig, is injected into a series of animals. About three weeks after infection, the sensitiveness of the guinea pigs to tuberculin is determined by giving one guinea pig daily an intra-abdominal injection of 0.375 gm. O. T. per 500 gm. body weight. When the sensitiveness of the animals has reached a degree at which this dose causes death within twenty-four hours, 2 guinea pigs are injected daily with 0.25 gm. O. T. per 500 gm. weight. The day after it is found that this dose kills one at least of the two guinea pigs within twenty-four hours, 6 guinea pigs are injected, each with 0.25 gm. O. T. per 500 gm. weight, and, if 4 or more pigs die in twenty-four hours, the remaining infected pigs are regarded as being sufficiently sensitive for determining the potency of the tuberculin of unknown strength. When the guinea pigs have reached the desired sensitiveness, as determined by the method just described, they are then divided into groups of six. One group is used to each sample of tuberculin the potency of which is to be determined. Each animal of the group receives an intraäbdominal injection of 0.25 gm. O. T. to be tested per 500 gm. body weight. As a control, another group of 6 guinea pigs is injected, each with an equal amount of the standard tuberculin, and two nontuberculous guinea pigs are injected with the tuberculin which is being tested, in order to make certain that the tuberculin contains no excessive amount of toxic material and no viable tubercle bacilli.

In order to pass the test, it is required that the tuberculin should, within twenty-four hours, kill at least half as many of the test guinea pigs as are killed in the control group injected with the standard tuberculin,

and that the animals should show, at autopsy, the characteristic lesions found when tuberculous animals succumb to lethal doses of tuberculin.

Borrel (6) observed that while an animal might prove to be refractory to subcutaneous injections of alkaloids or bacterial toxins, such was not the case if the alkaloid or toxin was injected intracerebrally. Von Lingelsheim (7) utilized the findings of Borrel, suggesting this method as the basis for the standardization of tuberculin. Varying amounts of the tuberculin, precipitated by means of alcohol, were injected intracerebrally into a group of normal guinea pigs, while under the influence of an anesthetic, and the minimal lethal dose was thus determined.

Borrel (6), as well as Marie and Tiffenau (8), obtained results apparently in accord with these findings. Neufeld (9) found, however, that the reaction is not specific, and that the same results could be obtained from the alcohol precipitate of sterile glycerine broth, or from sodium sulphate or other salts. The method suggested by von Lingelsheim has never been generally accepted as a dependable one for the purpose of standardization.

The present day method of determining the potency of tuberculin is unsatisfactory, inasmuch as a large number of animals is required and these vary in their degree of sensitiveness to tuberculin and in their resistance to the tubercle bacillus, as is evidenced by the rate and extent of development of the tuberculous process. Because of these individual variations among guinea pigs, the results obtained with any specimen of tuberculin must prove to be somewhat irregular and variable. The method is obviously time-consuming and expensive.

Roemer (10), utilizing the intracutaneous method of Mantoux (11) (12) in cattle, found that the intracutaneous injection of 0.05 cc. of tuberculin, diluted with an equal amount of salt solution, yielded results which corresponded with those obtained by the subcutaneous injection of 5 cc. of 10 per cent tuberculin.

In order to determine the degree of the reaction Roemer measured the thickness of 5 cm. of the shaved hide before and after the injection. The reaction became manifest within twenty-four hours, reached its height after forty-eight hours and, within four to six days, had disappeared. Cattle, the hides of which showed an increase in thickness of 0.4 cm., were considered negative, 0.5 to 0.7 cm. doubtful, while all those showing an increase of 0.7 cm. and more were considered positive. A series of normal guinea pigs, guinea pigs injected three months pre-

viously with large doses of killed tubercle bacilli, and guinea pigs injected with a virulent culture of tubercle bacilli and showing all the evidences of a progressive tuberculosis, were tested by means of the intracutaneous method. For this purpose 0.02 cc. of tuberculin was utilized. Of the normal guinea pigs none showed any local or general reaction. Of the animals treated with killed cultures of tubercle bacilli about half showed a slight local reaction, while of the tuberculous animals all but one reacted with a marked local reaction as well as with a marked rise of temperature. The guinea pig which did not react was suffering from advanced tuberculosis and died five days later. Roemer (13) undertook a more systematic study of the intracutaneous reaction in guinea pigs and found that, the greater the dose and the virulence of the culture used, the more severe was the reaction and the shorter the time between the time of injection and the fully developed sensitive state. Furthermore, the intensity and sensitiveness of the reaction are in direct proportion to the rate and extent of the tuberculous process. By using a large dose of a virulent culture, Roemer found that the infected guinea pigs gave well marked intracutaneous reactions twenty-one days after infection.

The results of our studies on the relationship existing between the intensity of the reaction and the rapidity of onset of sensitiveness on the one hand and the dosage and virulence of the culture on the other hand corroborate the work of Roemer. We have found that guinea pigs infected subcutaneously or intraperitoneally with virulent cultures of tubercle bacilli gave distinctly positive intracutaneous reactions with tuberculin between eighteen and twenty-one days after infection.

The reaction to the intracutaneous injection of tuberculin is very definite in the guinea pigs, and one may easily use this method to ascertain the potency of tuberculin by injecting in adjacent places equal amounts of a tuberculin of unknown potency and a standard product. For this purpose, we have found that light colored guinea pigs show sharper reactions than do the darker animals.

To remove the hair from the animals, we have employed a depilatory made by mixing barium sulphide with a satuated solution of sodium sulphide, and adding to this mixture the white of egg and water until it assumed a paste-like consistence. The hair over the abdomen and the lower part of the thorax is first cut short with scissors. The depilatory is then applied and permitted to remain for a short time (about one to two minutes) when it is scraped off and the part washed thoroughly with running water. The danger of irritation to the parts is minimized by

the addition of egg albumen to the paste and by the removal of the depilatory as soon as possible. We have found it advisable to prepare the animals twenty-four to forty-eight hours before injecting them, so that any irritation of the skin will have subsided.

We find that a Luer type glass syringe of the "tuberculin" model is most satisfactory for this work. The needle should be fine, gauge 26, and platinum is our preference. The needle should be sharpened so that the point is very short and sharp. Roughness of the needle has leakage as a consequence. Two-tenths cubic centimetre is the maximum of fluid to be injected in one place at one time. One-tenth cubic centimetre is a better amount. Test doses can be adjusted to meet this limitation.

By means of the intracutaneous reaction we have determined the potency of six different samples of tuberculin, five of which were furnished to us by different manufacturers of biological preparations and one by the United States Bureau of Animal Industry.

Forty guinea pigs, ranging in weight from 200 to 300 gm. were injected intraperitoneally with 0.1 mgm. of tubercle bacilli. Virulent cultures of the bovine type were used. Eighteen days later four of the animals, when injected intracutaneously with 0.02 mgm. of tuberculin of known quality, gave a well marked reaction which indicated that the animals were sensitive. Three days later, or twenty-one days after infection, the remaining 36 animals were divided into six groups of 6 each, and the potency of a different tuberculin tested on each different group. All 6 pigs in all groups received the following amounts of tuberculin: 0.02, 0.01, 0.005, 0.002, 0.001 mgm., which were brought up to a total volume of 0.1 cc. and injected intracutaneously by means of a tuberculin syringe and a short 26 gauge needle.

Readings were made 24, 48 and 72 hours after the injections, but only those made after 48 hours are tabulated, since the reaction reaches its maximum at that time. Degrees of reaction are designated by the figures 1, 2, 3, and 4. (4) indicates a very marked reaction, as evidenced by marked redness, edema, necrosis or hemorrhage. (3) and (2) indicate reactions of a less marked redness and edema, depending upon their degree of severity. (1) designates those reactions which show slight redness as well as slight but distinct thickening at the site of injection. There appeared to be no relationship between loss of weight and sensitiveness to the intracutaneous injection of tuberculin. In order to control further the potency of the different samples of tuberculin and to

TABLE 1

NUMBER	21 DAYS AFTER INFECTION	25 DAYS AFTER INFECTION
	Tuberculin Number 1	Tuberculin Number 4
1896	0.001	0.02
1899	Died 24 hours, typical tuberculin findings	
1901	0.001	Neg.
1905	0.001	Neg.
1906	0.001	Neg.
1907	0.001	Neg.
	Tuberculin Number 4	Tuberculin Number 1
1852	Neg.	0.001
1915	Neg.	0.005
1920	Neg.	0.02
1922	0.02	0.002
1924	Neg.	0.005
1926	Neg.	0.005
	Tuberculin Number 2	Tuberculin Number 3
1903	0.001	0.005
1935	0.002	0.005
1936	0.01 Died in 96 hours	
1937	0.005 Died in 96 hours	
1939	0.005	0.02
1941	0.002	Neg.
	Tuberculin Number 3	Tuberculin Number 2
1851	0.005	0.005
1908	0.01	0.005
1912	0.02	0.005
1913	0.005	0.005
1914	0.01	0.01
1916	0.01	0.01
	Tuberculin Number 5	Tuberculin Number 6
1925	0.02	0.02
1928	0.001	0.002
1934	0.005	0.005
1938	0.002	0.002
1940	0.001	0.002
	Tuberculin Number 6	Tuberculin Number 5
1933	0.001	0.02
1853	0.005	0.005
1927	0.02	0.02
1929	0.005	0.01
1930	0.005	0.005
1932	0.001	0.005

prove that the difference in the reactions was due to the potency of the tuberculin and not to the sensitiveness of any group of animals, we reinjected the guinea pigs twenty-five days after infection and so arranged them that a group which had yielded a strong reaction with one sample of tuberculin was injected the second time with a tuberculin which had given only a slight reaction or no reaction with another group.

Table 1 gives a summary of the results obtained with these groups of animals, forty-eight hours after the injection of tuberculin. The amounts noted in this table gave a plus 1 reaction. Tuberculin number 2 is the standard of the U. S. Bureau of Animal Industry.

A study of the table reveals a considerable difference in the reactions produced by the different samples of tuberculin under consideration. It is also evident that the individual variation in reaction of the animals is wide. If we consider only the tests done on the twenty-first day, it appears that tuberculin number 1 is the most potent, and that number 4 has a very slight value. The others are intermediate. If it were our purpose to furnish the complete specifications for a method of testing tuberculin we should have felt obliged to standardize these preparations by the accepted method. Since this would necessarily be repeated by those responsible for the adoption of such a test we have felt that this would be an unnecessary duplication if completed by us. We feel that the results presented are sufficient to call attention again to Roemer's work as a probable basis for a less expensive and perhaps more accurate method of testing the potency of tuberculin.

It will be noted that, in a general way, the results obtained on the twenty-fifth day follow the order of those of the twenty-first day, but that the preparations seem to be less active. It is most likely that this represents a measure of desensitization as the result of the previous test. Allowing for this difference, it may be considered that the varying potency displayed by the first test is fully confirmed by the second. It may be presumed that in practice sensitiveness may after the lapse of ten or more days, instead of four, be recovered or increased and that further economy may be effected through the repeated use of animals.

SUMMARY AND CONCLUSIONS

Roemer's work on the intracutaneous tuberculin test in guinea pigs does, we believe, form the basis for an improved method of standardizing, tuberculin. If the test animals are infected with 0.1 mgm. of a virulent

culture intraperitoneally they may be fit for use (sufficiently sensitive, that is) in three weeks. In other instances this point may take longer to reach. We have not attempted to elaborate a standard method of testing tuberculin, but do call renewed attention to the skin reaction in the belief that all of the essential facts are at hand which would permit of a revised method which might perhaps be economical of time and animals and which might in practice be more accurate than that at present in use.

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IMMUNOLOGICAL STUDIES IN TUBERCULOSIS[1,2]

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Koch, early in his studies on the resistance of animals to tubercle bacillus infection, made the following observation which has become one of the fundamental premises of all subsequent immunological studies on this disease.

When a normal guinea pig was inoculated subcutaneously with living virulent tubercle bacilli, there was no immediate localized reaction following the inoculation, with the exception of a very small traumatic one which soon disappeared. For about 10 days nothing happened at the site of inoculation. At the end of about 2 weeks, a small papule appeared, increasing in size and inflammatory appearance day after day, and, in about 15 to 20 days, developing into a definite mass. This mass gradually broke down to form an ulcer, which increased in size and never healed. On the other hand, if an inoculation similar to the one mentioned above was made in an animal which had already been infected, an entirely different train of occurrences supervened. Within twenty to twenty-four hours, erythema and induration appeared in the area surrounding the site of inoculation, which gradually increased in size and severity for forty-eight to seventy-two hours, after that gradually subsiding in the course of the following five, six or seven days. In the infected animal, as in the normal one, a papule was left at the site of inoculation, which gradually developed into a mass which eventually ulcerated, but, in contrast to the analogous mass in the normal animal, it tended to decrease in size and go on finally to complete healing.

Practically all students of tuberculosis agree on the following points: (1) As soon as a tuberculous animal becomes hypersensitive to the intracutaneous skin test, a certain degree of immunity has been established and that, at such a stage, it cannot be readily reinfected; (2) that such resistance is not absolute, but fluctuates considerably, and

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runs parallel with hypersensitiveness. Such have been the observations on experimental animals.

It is not unlikely that the same facts hold true for the human race. The majority of people who have tuberculosis pass through a long life and react positively to intracutaneous tests. Yet, although many of them may harbor living tubercle bacilli in localized foci, they may never give symptoms of specific illness.

Pirquet introduced a new method of determining the hypersensitiveness observed by Koch. It indicates the presence of tubercle, whether healed, latent, or active.

Twenty years ago Trudeau, Baldwin and Kinghorn (1) were convinced that the phenomenon of the tuberculin reaction had an important bearing on immunity in the experimental tuberculosis of guinea pigs. By a tuberculin reaction they meant only the systemic reaction with fever, since at this time the intracutaneous test was unknown.

In 1908, Mantoux and Moussu (2), and again Mantoux (3), described the intracutaneous reaction in guinea pigs. This made possible the attempt to estimate the severity of the infection, the progress of the disease, and also the degree of immunity by a study of skin hypersensitiveness in small animals.

The study of hypersensitiveness to tuberculin was taken up by many observers. Some of the most important work along these lines up to 1916 was done by Römer, Baldwin and Krause. Römer (4) concluded that the intensity of the tuberculin reaction varied directly with the extent and progress of the tuberculous process. Baldwin (5) was more interested in the conditions under which skin sensitiveness might occur in guinea pigs. He inoculated them with products of tubercle bacilli, but could obtain no positive tuberculin reaction unless he produced tubercle by the inoculation of either living or dead bacilli. He came to the conclusion that only the presence of anatomic tubercle endows an animal with the capacity of reacting intracutaneously to tubercle bacillus products. Krause (6) made a thorough study of animals infected with tubercle bacilli, H 37, a strain of human origin and of moderate virulence, which in guinea pigs always produced progressive tuberculosis, involving the viscera and ultimately leading to the death of the animal. He also used another strain, R 1, which had lost virulence to such a degree that it produced only regional lymphatic node tuberculosis, after subcutaneous inoculation, and but very slight or no gross visceral lesions. Krause drew the following conclusions: (1) There is no cutaneous hypersen-

sitiveness without a focus (tubercle); (2) this hypersensitiveness appears coincident with the establishment of the focus; (3) it diminishes with the healing of the focus; (4) it varies directly with the intensity of the disease, which, in turn, is dependent upon the virulence of the inoculated microörganisms.

McJunkin (7), in a recent publication, describes the production of skin hypersensitiveness in guinea pigs by subcutaneous inoculation of filtrates of peritoneal exudates. This exudate he obtained from the peritoneum of tuberculous guinea pigs, dead from the reaction of a second intraperitoneal inoculation with living virulent tubercle bacilli mixed with gelatin. Selter (8) more recently emphatically states that skin sensitiveness is produced only by infection with living tubercle bacilli.

In considering immunological conditions in tuberculosis, a clear conception of the differentiation between anaphylaxis to tuberculoprotein and tuberculin hypersensitiveness must be kept in mind. While the two phenomena may have a definite relationship, into the details of which we cannot enter at present, they are quite distinct in a number of important respects. An indication of this was first given by Baldwin who found that skin hypersensitiveness and true anaphylactic reactions did not necessarily run parallel. In a recent investigation, in which the isolated uterus method was employed, Zinsser (9) confirmed this, showing that while skin hypersensitiveness develops within eight or ten days after infection with living bacilli, true anaphylactic reactions of the uterus cannot be elicited in such animals for three to six weeks or longer, according to the degree of infection. Conversely, animals treated by 10 to 12 daily injections of considerable amounts of tubercle bacillus extracts did not become skin-hypersensitive at any time, but within about three weeks after the last of the series of injections they developed typical anaphylactic reactions. Thus, it was possible to obtain animals, by infection with living microörganisms, which for a long period showed only marked skin hypersensitiveness, but no anaphylaxis; while, by the injection of dead bacillary derivatives, anaphylaxis without skin sensitiveness could be produced. Moreover, in investigations that are not yet completed, a relationship between these two types of hypersensitiveness and the different constituents of the bacterial extracts was suggested. Skin hypersensitiveness, and probably other forms of tuberculin reaction seems thus to be particularly associated with infection, and have been thus far demonstrated only in infected animals by injection of materials

derived from the bacilli or by injections of living or dead bacilli themselves. The special conditions under which the injection of dead tubercle bacillus materials may produce skin reactions were incorporated in the communication by Zinsser mentioned above. In his general studies on the relationship of the tuberculin type of reaction to anaphylaxis, he pointed out that there were probably two kinds of skin reaction. One is the typical anaphylactic reaction illustrated by the urticarial wheal which develops immediately after the injection of small amounts of horse serum into horse-serum-sensitive human beings, and which may be elicited in protein-anaphylactic guinea pigs by similar intracutaneous injection. This reaction comes on immediately, is evanescent, and never leaves behind deep tissue injury, the skin returning to normal within a few hours to a day. The other, the tuberculin, typhoidin, mallein, etc., type of reaction, is not associated with what is generally spoken of as protein anaphylaxis; it does not appear for eight to twelve or more hours after injection; and it is accompanied by a much deeper injury of the tissues and a slower disappearance. This type is particularly associated with bacterial infection. Believing that the importance of Krause's observations concerning the parallelism between this type of skin sensitiveness and resistance was one of the important basic problems in the study of immunity to tuberculosis, Zinsser carried out a few preliminary experiments in which he attempted to produce skin hypersensitiveness by the injection of large amounts of the "nucleoprotein" fraction of tubercle bacillus extracts. These experiments, small in number and reported only in a preliminary way, were suggestive of the possibility that such attempts might be eventually successful, and it was this problem mainly which formed the point of departure for the studies included in this paper.

In the fall of 1921, at the Department of Bacteriology, Columbia University, we continued these experiments.

ANIMALS USED IN THE EXPERIMENTS

Young guinea pigs, of an average weight of 350 to 400 gm. and practically all white in color, were used in the experiments. After the intracutaneous control test was found to be negative, they were inoculated intraperitoneally. Personal attention was given to the care and feeding, and the weights were very carefully recorded. We shall see later in the different experiments that the condition of the animal proved to be one of the most important factors in successful sensitization.

PREPARATION OF MATERIAL FOR SENSITIZATION

The preparation of the materials used was that described by Zinsser and was prepared as follows:

Tubercle bacilli, of human type and of moderate virulence, which always produced progressive tuberculosis in guinea pigs, generally involving the viscera and ultimately leading to the death of the animals, were used. The bacilli were cultivated on peptone-veal broth containing 5 per cent glycerine for from four to five weeks. Such cultures were filtered through heavy filter paper, and the masses of tubercle bacilli, while still on the filter paper, were washed several times with sterile water until the filtrate no longer gave a precipitate with alcohol. The masses of tubercle bacilli were very carefully removed to an evaporating dish and dried *in vacuo* over sulphuric acid for five or six days while protected from light.

Five grams of these dry masses were put in a porcelain ball mill and pulverized from 2 to 3 weeks, and further treated as follows:

About 500 mgm. of the dry pulverized tubercle bacilli, H 37, were extracted with a solution of isotonic salt solution containing 0.2 per cent sodium hydrate. After the two had been well mixed, they were shaken in a mechanical shaker for four hours. They were then filtered through ordinary filter paper until perfectly clear, and later filtered through a Berkefeld filter. The reaction of this filtrate was about pH 8. This filtrate was then precipitated with 10 per cent acetic acid, added drop by drop, and centrifugated until no further precipitate appeared. The precipitate was redissolved in alkaline salt solution and precipitated a second time with 10 per cent acetic acid. After centrifugation, the precipitate was again dissolved in the alkaline salt solution and brought to about pH 7.6 by the addition of a few drops of 2 per cent acetic acid; the volume was made up to 20 cc. with the alkaline salt solution. No attempt was made to standardize it by weight of the dried precipitate. The acidulated filtrate from the first precipitation was boiled for about one minute to remove the coagulable protein and then filtered through a Berkefeld filter. The filtrate was not standardized. Throughout the experiments we shall speak of the former as *tuberculo-nucleoprotein* (*TNP*). The final filtrate which remained after the so called "nucleoprotein" and the coagulable proteins obtained by boiling in acid had been removed, we speak of as the *residue antigen*, since this is the term applied to this material from tubercle bacilli and to analogous substances obtained since then from other bacteria by Zinsser (9) and by Zinsser and Parker (10).

This preparation of the material, as described above, was used except in the first two experiments. The extracts used in these two experiments were not filtered through Berkefeld filters.

In all experiments the *nucleprotein* and the *residue* were obtained from approximately equal amounts of pulverized tubercle bacilli; but, later, more care was taken in the amounts of material used from which the two substances were extracted. It was found that the residue diminished slightly in strength when left in the refrigerator, and for this reason every few days a new preparation was made. The *nucleoprotein*, just before use, was diluted 1 part in 10, with salt solution (normal); the *residue antigen* was used in undiluted form.

Pulverized tubercle bacilli (500 mgm.); 200 cc. of 0.02 per cent NaOH in 0.85 per cent NaCl solution; extraction by shaking for four hours.

1.—filtered through filter paper

2.—filtered through Berkefeld filter

The filtrate precipitated with acetic acid.

Precipitate collected by centrifugation.

Precipitate redissolved in alkaline salt solution (same as in first step); again precipitated with 10 per cent acetic acid; centrifugated. The precipitate was again taken up in 20 cc. of alkaline salt solution. This is designated in the present study as *nucleoprotein;* when used for the skin test it was diluted 1 in 10 parts isotonic saline solution.

Filtrate boiled for one minute to remove the coagulable protein, then filtered through Berkefeld filter.

The filtrate contains, among other substances, primary and secondary proteoses, peptones, polypeptids and amino acids. This is designated as *residue antigen*, and was used for the skin tests in an undiluted form.

MATERIAL USED FOR THE INTRACUTANEOUS TEST

Throughout this study the following substances were used for the intracutaneous tests: In the first two experiments, 10 per cent old tuberculin, 10 per cent tuberculo-nucleoprotein and undiluted *residue antigen.*

The intracutaneous test was made with a small glass hypodermic syringe and a 26-gauge needle, ½ inch long. The amount of substance for the skin test was not measured but the size of the bleb was made uniformly about 3 mm. The skin test was done alternately on the right and left sides of the abdomen. After plucking and shaving the animal, the different substances used for comparison were injected about 5 cm. apart on the same side. The results of the test were recorded in the first two experiments only at the end of the twenty-fourth hour, while in the later experiments a careful record was also made at the end of the forty-eighth hour. A positive skin reaction was recorded when at the end of the twenty-fourth hour an erythema showed with induration. Pale erythema without induration was recorded as more or less questionable.

Series I: Started October 4, 1922. Six guinea pigs (A205, A206, A207 A208, A209, A210) 260 to 390 gm. The tuberculo-nucleoprotein was prepared as above, but filtered only through wet filter paper, and intraperitoneal injections were made as follows:

2 g. p. every other day—2 cc. each, total of 6 cc.
2 " " " 2 cc. " " 10 cc.
2 " " " 2 cc. " " 12 cc.

(Tabulation is here omitted because of the uniformly negative results.)

Intracutaneous tests with old tuberculin, nucleoprotein and residue, on October 17, 21, 25, 1921, and February 19, 1922.

Results summarized: All tests were negative except on guinea pig A207, which gave a positive test, ++ *O.T.*, +++ (*TNP*), and ++ (*Residue*), 138 days from the beginning of the experiment, and 130 days since the last injection.

The animal was killed on the same day.

The autopsies on these guinea pigs, done at various intervals, were entirely negative for macroscopic tuberculosis. Guinea pig A207 which had given a positive reaction to all antigens was killed on February 19th, 138 days after the beginning of the experiment. It showed a small, grayish, tubercle-like mass about 0.5 mm. in size on the liver. This spot, together with surrounding tissues, was ground up in salt solution and inoculated into guinea pig A127, weight 360 gm. This was done on February 19, and on April 11 skin tests on this guinea pig were negative, and autopsy showed no macroscopic tuberculosis.

A typical autopsy in this series was as follows:

October 30, 1921: A210 was found dead. *Autopsy:* Peritoneum was much congested. Spleen, liver and lungs were normal. Mesenteric, inguinal and bronchial nodes were normal.

Series II: In this series only 3 guinea pigs were used. Injections of 1 cc. of the nucleoprotein, filtered through paper, were given every three or four days, until 5 cc. had been administered.

Intracutaneous tests were done on these animals at varying intervals with three preparations, old tuberculin, nucleoprotein solutions and residue antigen.

The results may be briefly summarized, in that positive tests were obtained on all of these animals on the 25th, 35th and 46th days after the beginning of the experiment and the 13th, 23d, 34th days after the last injection, respectively.

Autopsies on these animals showed no macroscopic tuberculosis.

Series III: In order to avoid the possible criticism that some tubercle bacilli which had not been pulverized might have been responsible for such hypersensitiveness, all our preparations in this series and the ones hereafter recorded, were filtered through Berkefeld filters. It is well known that as few as 10 tubercle bacilli may produce tuberculosis in guinea pigs. This observation was made some years ago by the author, and we believe that probably much smaller numbers of tubercle bacilli can produce anatomic tubercle without evidence of macroscopic tubercles. Our material was obtained by the same method as employed in the second experiment. After it had been shaken and extracted with alkaline salt solution, it was left in the icebox overnight, then filtered through filter paper until clear, and then passed through small type *V* Berkefeld filters. It was precipitated with acetic acid, centrifugated, redissolved and then this procedure was repeated. The extract from 200 mgm. of the tubercle bacilli was then taken up in 20 cc. alkaline salt solution, made up to pH 7.6 and used for inoculation. For every inoculation a fresh preparation was used.

By this method of filtration we noticed that large amounts of the nucleoprotein fraction were lost during the filtration through the Berkefeld filter. So for this reason we used 2 cc. for each guinea pig. Further details of this experiment are to be found in table 1.

TABLE 1

Guinea pigs sensitized with tuberculo-nucleoprotein

Series III

Guinea-pig No.	Injection of antigen.							Material for the skin test.	Intracutaneous test.								
	Nov. 19, 1921.	Nov. 21, 1921.	Nov. 23, 1921.	Nov. 25, 1921.	Nov. 29, 1921.	Dec. 1, 1921.	Dec. 3, 1921.		Dec. 4, 1921.	Dec. 11, 1921.	Dec. 20, 1921.	Jan. 4, 1922.	Feb. 17, 1922.	Feb. 24, 1922.	Mar. 23, 1922.	May 8, 1922.	
A 247	2 c.c.	2 c.c.	..	..	2 c.c.	2 c.c.	..	O.T.	O	++	++	++	++	..	..	..	Feb. 20, 1922: Killed; see autopsy details and sub-injection.
								T.N.P.	+−++	++	+++?	++	+	..	..	..	
								Residue	O	+	+	+	O	..	..	..	
A 248	2 c.c.	2 c.c.	..	..	2 c.c.	2 c.c.	..	O.T.	O	++	++	++	..	..	..	..	Feb. 4, 1922: This animal was used for another experiment.
								T.N.P.	+−++	++	++	++	..	..	..	..	
								Residue	O	++	++	+−++	..	..	..	..	
A 249	2 c.c.	2 c.c.	2 c.c.	5 c.c.	2 c.c.	2 c.c.	2 c.c.	O.T.	O	++?	++	++	++	++	..	..	Feb. 26, 1922: Found dead; no macroscopical evidence of tuberculosis.
								T.N.P.	O	+−++?	++	++	++	++	..	..	
								Residue	O	O	O−+	+	+	+	..	..	
A 250	2 c.c.	2 c.c.	2 c.c.	5 c.c.	2 c.c.	2 c.c.	2 c.c.	O.T.	O	O	..	..	..	..	..	..	Dec. 19, 1921: Found dead; no macroscopical evidence of tuberculosis.
								T.N.P.	O	O	..	..	..	..	..	..	
								Residue	O	O	..	..	..	..	..	..	
A 251	2 c.c.	2 c.c.	2 c.c.	5 c.c.	2 c.c.	..	..	..	..	..	..	..	..	..	..	..	Nov. 30, 1921: Found dead; no macroscopical evidence of tuberculosis.
A 252	2 c.c.	2 c.c.	2 c.c.	5 c.c.	2 c.c.	2 c.c.	2 c.c.	O.T.	O	O	+	++	++	+−++	10 x 10	O	July 10, 1922: Found dead.
								T.N.P.	O	O	+	++	++	++	10 x 10		
								Residue	O	O	O	+	+	O	6 x 6	O	

AUTOPSY OF THE ANIMALS

Guinea pig A251 died November 30, 1921. No macroscopic tuberculosis.

Guinea pig A250 died December 19, 1921. Apparently no tuberculosis. All organs and glands normal.

Guinea pig A248 was used on February 4, 1922, for superinfection.

Guinea pig A247 killed February 20, 1922. Lungs, liver and spleen normal, mesenteric nodes normal, bronchial nodes slightly enlarged. Smears showed no tuberculosis. *Subinjection:* The suspected material from the animal was ground up with salt solution and injected into a healthy guinea pig, A1127. March 14th, large masses at the site of injection. This pus was examined for acid-fast bacilli and for other microörganisms, resulting in negative findings. On August 11, 1922, skin test was made on guinea pig A1127. Twenty-four hours later reaction was negative. The animal was killed August 12, 1922. *Autopsy:* No evidence of tuberculosis.

Guinea pig A249 killed February 26, 1922. Autopsy showed no macroscopic tubercles. All organs normal.

July 10, 1922, guinea pig A252 was found dead. *Autopsy:* Weight 510 gm., superficial inguinal nodes, peritoneum, liver and lungs normal. Bronchial and cervical nodes slightly enlarged. The suspected material was ground with salt solution and injected into guinea pig 31, weight 432 gm. Thirty days after the subinjection, skin test was done on guinea pig 31, using 5 per cent *O.T.*, and *residue antigen* for eliciting reaction. At the 24th hour reaction was negative. The animal was killed two days later, and showed no macroscopic tuberculosis.

Summary of Series III: It is evident that the guinea pigs which received 17 cc. of the preparation did not react any more strongly than the one which received less than half of this amount. It will also be noted from the tables that ninety days from the beginning of the inoculations, the skin reaction became weaker. It is very much regretted that we did not inoculate the organs of the dead and killed animals, which looked apparently normal. Never in one instance did our reactions in the treated animal reach the same degree of intensity as that obtained in the tuberculous animals.

Series IV: The same method of preparing the tuberculo-nucleoprotein as in the preceding experiments was used in this experiment, except that a larger amount of pulverized bacilli (200 mgm.) was used. It was shaken as before and filtered through type *V* Berkefeld filter, etc. For this experiment large white guinea pigs, 400 to 500 gm., were used. Further details of this experiment are to be found in table 2.

TABLE 2

Guinea pigs sensitized with tuberculo-nucleoprotein

Series IV

Guinea-pig No.	Injection of antigen.					Material for the skin test.	Intracutaneous test.											
	Feb. 3, 1922.	Feb. 8, 1922.	Feb. 27, 1922.	Mar. 10, 1922.	Mar. 30, 1922.		Feb. 10, 1922.	Feb. 14, 1922.	Feb. 24, 1922.	Mar. 8, 1922.	Mar. 16, 1922.	Mar. 23, 1922.	April 3, 1922.	April 17, 1922.	May 8, 1922.	May 23, 1922.	June 9, 1922.	
A 289	2 c.c.	2 c.c.	2 c.c.	2 c.c.	2 c.c.	O.T.	?	+	+	++	+	6 x 6	6 x 6	O	O	10 x 10	..	June 3, 1922: Found dead; see autopsy details and subinjection.
						T.N.P.	++	++	O	++	++	6 x 6	6 x 6	O	..	..	..	
						Residue	?	+-++	O	+	+	O	O	O	O	5 x 5	..	
A 307	2 c.c.	2 c.c.	2 c.c.	2 c.c.	..	O.T.	..	O	+	+	+	O	..	..	..	..	..	Mar. 30, 1922: Found dead; no macroscopical evidence of tuberculosis.
						T.N.P.	..	++	+	++	+	O	..	..	..	..	..	
						Residue	..	O	O	+	+	O	..	..	..	..	..	
A 310	2 c.c.	2 c.c.	2 c.c.	2 c.c.	..	O.T.	..	++	++	+++	+++	20 x 20	20 x 20	15 x 15	10 x 10	10 x 10	..	June 13, 1922: Found dead; see autopsy details and subinjection.
						T.N.P.	..	+++	+	+++	++++	20 x 20	20 x 20	15 x 15	..	..	..	
						Residue	..	++	+	+-++	+++	6 x 6	7 x 7	5 x 5	8 x 8	5 x 5	..	
A 312	2 c.c.	2 c.c.	2 c.c.	..	..	O.T.	..	++	O	..	..	..	..	..	..	..	..	Mar. 1, 1922: Found dead; very much emaciated; weight, 250 gm.; no microscopical evidence of tuberculosis.
						T.N.P.	..	+++	O	..	..	..	..	..	..	..	..	
						Residue	..	++	O	..	..	..	..	..	..	..	..	
A 313	2 c.c.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	Feb. 6, 1922: Found dead; peritoneum very much congested; no evidence of tuberculosis; cultures and smears were negative.
A 315	2 c.c.	2 c.c.	2 c.c.	..	..	O.T.	..	O	++?	+++	+++	15 x 15	15 x 15	10 x 10	O	O	O	Oct. 17, 1922: Killed by chloroforming; see autopsy details and subinjection.
						T.N.P.	..	++	++	+++	+++	15 x 15	15 x 15	10 x 10	..	..	..	
						Residue	..	O	O	++	++	5 x 5	5 x 5	5 x 5	O	O	O	

June 3, 1922: Guinea pig A289 was found dead. Weight 325 gm. *Autopsy:* Superficial and deep inguinal and iliac nodes normal. Spleen of normal size and slightly congested, probably postmortem. Lungs very much congested and hemorrhagic; liver, bronchial and tracheal nodes normal. No evidence of tuberculosis. The bronchial and cervical nodes and spleen were ground with salt solution and injected subcutaneously into guinea pig 100, weight 352 gm.

July 24, 1922: Intracutaneous test done on guinea pig 100, using *O.T.* and *residue antigen. Result:* Negative. This animal was killed on August 5, 1922. Autopsy showed no tuberculosis.

July 13, 1922: Guinea pig A310 was found dead, weight 536 gm. Superficial and deep inguinal nodes normal; peritoneum, liver and lungs normal. Spleen enlarged, 18 × 30 mm., nodular. No acid-fast bacilli in smears prepared from these nodules. The portion of the spleen was ground up with salt solution and injected subcutaneously into guinea pig 102, weight 320 gm. One other portion was inoculated on gentian violet media. On July 24 and August 4, 1922, skin test was done on this subinjected guinea pig. The animal responded negatively to 5 per cent old tuberculin and residue antigen at the twenty-fourth hour.

August 5, 1922: Guinea pig 102 was killed. *Autopsy:* No macroscopic tuberculosis.

October 17, 1922: The remaining guinea pig A315, weight 385 gm., was chloroformed. *Autopsy:* Emaciated; peritoneum apparently normal; spleen slightly enlarged; liver, lungs, superficial inguinal and bronchial nodes normal. Cervical nodes much enlarged, hypertrophic, no caseation. One of the mesenteric nodes hypertrophic but not caseous. A small portion of the spleen and lymph nodes was fixed in Zenker's fluid and the following are the histological findings reported by Dr. Gardner, Saranac Laboratory:

Spleen: The lesions are found within the splenic pulp and are of a chronic nature. They consist of dense masses of fibroblasts and endothelial cells without the regular separation into zones. Leucocytic invasion of the central portion is regular and not marked. An occasional giant cell (not a megalokareocyte) is found in bloodvessels adjoining the lesion. There is also some fibrosis about the germinal centre. A Ziehl-Neelsen stain failed to reveal any acid-fast organisms. The small bodies resembling bacteria in lymphatics could not be determined.

Lymph Nodes: In the medulla are very large conglomerate lesions with very intense leucocytic infiltration. No acid-fast bacilli could be found.

Another portion of the suspected material was ground in a mortar and injected into guinea pig 140, weight 423 gm.

February 14, 1923: Guinea pig 140, weight 560 gm. Intracutaneous test with old tuberculin and residue antigen. *Result.* At twenty-four hours negative.

February 15, 1923: Killed. No tuberculosis at autopsy.

No definite conclusions can be drawn from the above first four series of nucleoprotein-sensitized guinea pigs. It seems that in many cases we succeeded in producing skin reactiveness in guinea pigs treated with the nucleoprotein fraction. This skin reactivity appeared after various intervals between series of injections, but in most instances showed a tendency to fade with relative speed. However, although we filtered through Berkefeld candles, in the two series, and although we never found living tubercle bacilli either by culture or by transfer to new animals, in the animals which showed such reactivity, nevertheless some of these animals showed small visible lesions in the liver, and in some instances in the spleen. These lesions consisted of minute grayish specks, which might be interpreted as meaning a temporary lodgment of whole dead or living bacilli which may have passed through our filters.

In consequence, we determined to elaborate these experiments as follows:

Series V.—This experiment was carried out by methods identical with the preceding, except that a considerably larger amount of tubercle bacillus powder was used, and the filters used for filtration of the extracts were Berkefeld *W*, which are less permeable than those previously used.

In this series 12 guinea pigs were used, and from 7 to 11 injections of the nucleoprotein were given to them between March 11 and July 24.

Intracutaneous tests with the three preparations previously used were done at varying intervals, beginning April 3, until February 15 of the following year. Since the tabulation of this experiment is a very large and complicated one, it will suffice to summarize the results as follows: In almost all of the guinea pigs skin reactions were obtained, at one time or another, in the course of the experiment. As a general rule, the reactions did not exceed 5 × 8 mm., and never 12 × 12 mm. All of them were very transient, disappearing almost completely within twenty-four to forty-eight hours. None of the guinea pigs had given any reaction to 10 per cent old tuberculin, before use in the experiment. In some of the animals the reactions were obtained on only one or two occasions; in others they persisted for longer periods.

These guinea pigs were kept for observation for long periods, and 8 of them eventually died between May, 1922, and February, 1923. Some died in transit to Saranac Lake; others died of intercurrent disease,

probably gastroenteritis. On 7 of them careful autopsies were performed. All of these 7 had shown some kind of reaction at one time or another. In 2 only small grayish spots were found. Histological examination of the suspected lesions in one of these 2 guinea pigs, A125, which was killed 340 days from the beginning of the experiment was made by Dr. Gardner, who reported as follows:

Spleen: The capsule shows focal thickening by vascular fibrous tissue, within which are more or less circumscribed nodules, the largest attaining a diameter of nearly 1 mm. The nodules are composed of peripheral, concentrically arranged fibroblasts containing a few small capillaries. Within this outer coat are closely packed masses of large endothelial cells and an occasional giant cell (Langhans type). No blood vessels are found. The centre of the larger nodules is occupied by a mass of polymorphonuclear leucocytes, which in some instances have undergone necrosis. In the adjoining splenic pulp, in the case of each nodule, there is found a perivascular lymphatic, distended with a granular material resembling very minute bacteria, but with a magnification of 1500 diameters these could not be resolved to determine their form. The spleen pulp shows prominent germinal centres which show very occasional mitotic figures. In the sinuses are large numbers of poly- and mononuclear eosinophiles.

Liver: Shows no abnormality.

These lesions, while they superficially resemble tubercles, are not typical of a primary infection with tubercle bacilli. They rather suggest the mixed proliferative and exudative reaction found in reinfection with this microörganism.

Transfer of tissue to other guinea pigs never resulted in tuberculosis.

Series VI: The extract was filtered through a Berkefeld filter, type *W*.

Eighteen guinea pigs, from 378 to 548 gm., after preliminary intracutaneous tests, were injected intraperitoneally with tuberculo-nucleoprotein on August 4, 7, 12, 17, 23 and September 1, a total of 12 cc. in 6 injections. On August 29 and September 8 and 19, 1922, intracutaneous tests were done. The reactions were negative, except in 4 guinea pigs which gave mild atypical reactions.

Series VII: In this series 10 guinea pigs were injected as usual with tuberculo-nucleoprotein, of which, in every instance, the amount was determined; 500 mgm. of pulverized tubercle bacilli generally yielded 70 to 75 mgm. of acetic acid-precipitated substances. The injections were repeated four times, at intervals of four days, after which the skin tests

were done. Then 4 more injections were made a month later. The animals were injected eight times in all, with 2 cc. each time, a total of 16 cc. This made approximately 100 mgm. of tuberculo-nucleoprotein. The skin tests were done as usual. The results were practically all negative except in 3 guinea pigs, which gave moderate atypical reactions.

Series VIII: In the sixth and seventh experiments, Berkefeld filters, type *W*, were used. It is possible that some of our sensitizing substances were held back in these filters which, as mentioned above are less permeable. For this reason a new supply of type *V* which is much coarser was used in the following experiments.

The material used in this experiment was prepared in accordance with the usual method, but after the extraction was filtered through heavy paper until perfectly clear and divided into two fractions. Fraction *1* was precipitated with acetic acid, centrifugated only once and redissolved in alkaline salt solution, and in such a state was injected; while fraction *2* was filtered through a Berkefeld filter, type *V*, precipitated with acetic acid, only once centrifugated, and the precipitate redissolved in alkaline salt solution and used for injection.

Ten white guinea pigs were used in these experiments; 5 were injected intraperitoneally with Berkefeld filtrate, tuberculo-nucleoprotein, and the other 5 with the paper-filtered material.

January 23, 1923: At first three injections were made, one every other day, which covered a period of six days. After a rest of several days this was followed by two injections each day for two days; then a single injection each day, for 2 more days.

In the meantime intracutaneous tests were done and continued until March 6, 1923.

Result: In the first series, the animals treated with Berkefeld filtrate reacted definitely, but not typically, on the 31st day from the beginning of the experiment. The reaction, however, disappeared completely after the 24th hour. In the second group, those treated with paper-filtered material, two guinea pigs reacted definitely with a typical skin reaction, and this reaction persisted for forty-eight hours. These guinea pigs are still alive.

SUMMARY

In the preceding section we have reported 8 experiments, in which attempts were made to sensitize guinea pigs cutaneously by the injection of so called *nucleoprotein* fractions of extracts of powdered tubercle bacilli. The results obtained in these experiments may be summarized briefly as follows:

As was the case in the 4 guinea pigs reported previously by Zinsser, in the communication referred to, skin reactions were obtained with *O.T.*, *nucleoprotein*, and *residue material* in many of these guinea pigs at one time or another. There was no regularity in the appearance of the reactions, either as regards the quantities of nucleoprotein injected, or the times at which the reactions appeared. The reactions were similar to the tuberculin reactions that are to be observed in infected guinea pigs only in regard to the fact that a skin injection of tuberculin resulted in an area of localized inflammation within twelve to twenty-four hours. Unlike the typical tuberculin reaction, however, these areas usually disappeared within twenty-four to forty-eight hours and they never led to necrosis or hemorrhage, as is frequently seen in severe skin reactions in infected animals. Unlike animals that are infected with living tubercle bacilli, moreover, the period of sensitiveness to the skin reaction was not a protracted one, since most of the animals again became negative before death.

Interpretation of these results is difficult. We may assume, on the one hand, that with nucleoprotein we produced a minor degree of skin reactiveness to the tuberculin preparations, qualitatively like the sensitiveness of tuberculous animals, but quantitatively weaker. If this were the case, it might be due, on the one hand, to a sensitization by dead material from the tubercle bacilli, which produced, for a short time, the same condition that develops in a tuberculous animal, and we would be well started on our way to produce skin sensitiveness with soluble products of the tubercle bacillus.

On the other hand, in spite of our great care in attempting to exclude the possible presence of tubercle bacilli or particles of undissolved bodies of bacilli in the filtered fluids, the occasional small lesions noticed in some of the animals may indicate that, whenever we obtained a positive skin reaction, we were dealing with an animal not sensitized by soluble products, but one in which the lodgment of a minute amount of undissolved tubercle bacillus fragments may have produced skin

reactivity in the same way as Baldwin, in his original experiments with dead tubercle bacilli, showed could happen. The presence of living tubercle bacilli in these filtrates is also a possibility, but seems unlikely in view of the fact that none of these animals developed any extensive tuberculosis, the minute lesions described being the only morphological abnormalities noticed and the fact that most of these animals became negative after a short time.

These questions are difficult to decide, but some light may be thrown on them by the subsequent experiments, reported below, in which we show how readily tuberculin hypersensitiveness can be produced by the injection of dead tubercle bacilli.

SKIN SENSITIZATION OF GUINEA PIGS WITH DEAD TUBERCLE BACILLI

As already mentioned, Baldwin, in 1911, observed positive skin reactions in guinea pigs into which he had injected tubercle bacilli killed by heat. In view of these results, as well as our difficulty in producing any kind of skin hypersensitiveness with the nucleoprotein fraction, and in view of the apparent lack of confirmation in the literature of Baldwin's early results, it seemed important to investigate this point again. In consequence, we carried out a large series of experiments, in all of which guinea pigs were injected with a neutral suspension of tubercle bacilli that had been killed by various degrees of heat, and intracutaneous tests were performed with the three preparations used in the preceding experiments, namely, old tuberculin in 5 per cent dilution, nucleoprotein fraction, and the residue antigens described above.

In the first three experiments were used tubercle bacilli heated at from 60° to 80°C. for one hour. In all of these experiments, typical and extensive tuberculin reactions, in no way differing from reactions upon tuberculous control animals, were obtained in practically all of the guinea pigs used. Twenty treated guinea pigs and an equal number of normal and tuberculous controls were used in these first experiments, and there was no possibility of doubt that the injections of sufficient quantities of dead tubercle bacilli, that is, amounts varying from 12.5 to 37.5 mgm. of dry weight of tubercle bacilli, either at once or in successive injections, sufficed to produce in these guinea pigs definite and typical skin sensitiveness to all three of the materials used, sometimes within five days after the last injection, more frequently beginning at about the tenth day.

The detailed tabulations of these experiments are omitted because we believe that the principle is more convincingly covered by the following experiments in which the bacilli were heated at higher temperatures.

Having determined that heating to from 60° to 80°C. did not destroy the sensitizing properties of the dead tubercle bacilli, and wishing to exclude any possibility of having injected a few living bacilli, we now proceeded to the injection of tubercle bacilli suspended in salt solution and heated in a water bath to 100°C. for one hour.

Table 3 demonstrates that the injection of small amounts of tubercle bacilli heated for one hour at 100°C. produces, with considerable regularity, a typical tuberculin skin hypersensitiveness in guinea pigs. It is noticeable that after the injections of these, as well as those heated at a lower temperature in the preceding experiments, a mass may be felt which gradually increases and persists throughout the experiments. These masses, after intraperitoneal injection, are usually found adherent to the walls of the peritoneal cavity, surrounded by connective tissue, and contain a caseous mass, in which many acid-fast and non-acid-fast bacilli can be demonstrated. In most cases these masses persisted until the animals were killed or had died. In a few cases, the masses almost completely disappeared before the end of the experiment. The skin sensitiveness, as will be seen from the table, persisted three months or longer after the last injection of the tubercle bacilli, and there seemed to be a noticeable relationship between the persistence of the mass and the persistence of the sensitiveness. This is very closely confirmatory of the observations recorded by Baldwin in 1911. The curves in chart 1 illustrate the fluctuating relationship of the general condition of the guinea pigs' weights to the persistence of the skin sensitiveness and the relationship of both to the time after injection.

Although death from intercurrent gastroenteritis may occur in guinea pigs kept for observation for such long periods, we believe that none of the guinea pigs so injected died purely from the effects of the injection of the dead tubercle bacilli. In only a few of them did death occur within two or three weeks after the injection. Those that lived longer than this usually lived indefinitely, unless intercurrent infection carried them off. Autopsies were done on all that died and on a good many that were killed. In none was there any sign of tuberculosis other than that at the point of injection.

TABLE 3

Guinea pigs sensitized with tubercle bacilli killed at 100°C.

Intraperitoneal sensitization inoculation.				Intracutaneous test.															
Date.	June 26, 1922.	July 1, 1922.	July 5, 1922.	Date.	July 10, 1922.		July 18, 1922.		July 26, 1922.		Aug. 26, 1922.		Sept. 3, 1922.		Sept. 12, 1922.		Oct. 17, 1922.		Feb. 14, 1923.	
				Days.	15		23		31		62		70		79		114		234	
Days.	1	6	10	Time.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Guinea-pig No. 114	1 c.c.	1 c.c.	..	..	..	..	Died July 2, 1922: Congested peritoneum.													
Guinea-pig No. 115	1 c.c.	1 c.c.	1 c.c.	O.T.	10 x 10	5 x 5	15 x 15	5 x 5	15 x 15	12 x 12	15 x 20	15 x 15	20 x 20	15 x 15	15 x 12	10 x 10	15 x 15	5 x 5	10 x 10 pale	O
				Residue	15 x 15	10 x 10	15 x 15	10 x 10	15 x 15	10 x 10	15 x 15	10 x 10	15 x 15	10 x 10	10 x 10	5 x 5	18 x 18	5 x 5	10 x 10 pale	O
Guinea-pig No. 116	1 c.c.	1 c.c.	1 c.c.	O.T.	10 x 10	10 x 10	Died July 14, 1922: Autopsy: Many what appeared to be tubercles in the peritoneum; upon staining, many acid fast organisms were found; cultures were made on gentian-violet media; a small amount of the suspected material was inoculated into guinea-pig No. 84; weight, 360 gm.													
				Residue	8 x 10	8 x 8														
Guinea-pig No. 117	1 c.c.	1 c.c.	1 c.c.	O.T.	10 x 10	5 x 5	20 x 20	15 x 15	22 x 20	15 x 15	22 x 25	20 x 20	25 x 25	20 x 20	20 x 20	10 x 10	15 x 15	5 x 5	15 x 15 pale	O
				Residue	15 x 15	10 x 10	25 x 25	15 x 15	20 x 20	20 x 20	20 x 23	20 x 20	20 x 20	15 x 18	10 x 10	5 x 5	18 x 18	5 x 5	15 x 15 pale	O

TUBERCLE BACILLI KILLED AT 121°C., 15 POUNDS PRESSURE

In view of our invariable success in producing skin sensitiveness with tubercle bacilli killed at from 60° to 100°C., the following experiment, in which the bacilli were subjected to 121°C., at 15 pounds steam pressure, was performed.

Four guinea pigs were used, ranging in weight from 387 to 715 gm. After the preliminary skin test was shown to be negative, they were injected with a freshly prepared suspension of tubercle bacilli killed at

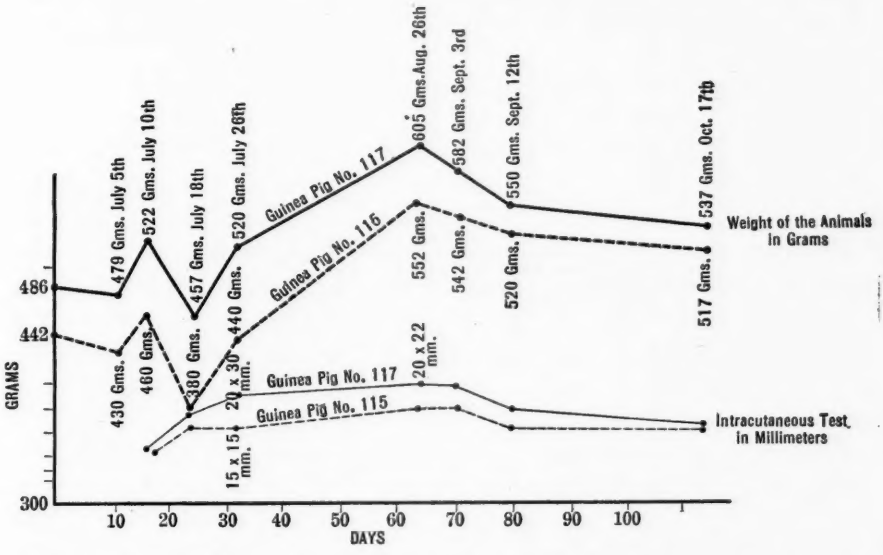

CHART 1. CORRELATION OF INTRACUTANEOUS TESTS WITH BODY WEIGHTS OF ANIMALS OF TABLE 3

121°C. for ½ hour. Each cubic centimetre of the suspension contained 12.5 mgm. of dead tubercle bacilli. All injections were made intraperitoneally. Table 4 shows the results. Some of these animals are still living at the day of writing, are gaining weight and are still giving moderately positive tests.

THE USE OF THE RESIDUE ANTIGENS FOR SENSITIZATION

In view of the fact that the residue antigens used by Zinsser could be successfully utilized for skin reactions in sensitive guinea pigs, it seemed important also to attempt to sensitize animals with this residual fraction

TABLE 4

Guinea pigs sensitized with tubercle bacilli killed at 121.3°C.

Intraperitoneal sensitization inoculation.				Intracutaneous test.									
Date.	Oct. 20, 1922.	Oct. 24, 1922.	Oct. 28, 1922.	Date.	Oct. 31, 1922.		Nov. 14, 1922.		Nov. 22, 1922.		Dec. 18, 1922.		Jan. 17, 1923.	
				Days.	11		25		33		59		89	
Days.	1	4	8	Time.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Guinea-pig No. 189	1 c.c.	1 c.c.	1 c.c.	O.T.	○	○	15 x 15	5 x 5	15 x 15	5 x 5	15 x 15	15 x 15	10 x 10	5 x 8
				Residue	○	○	8 x 8	○	20 x 20	10 x 10	15 x 15	15 x 15	10 x 10	8 x 8
Guinea-pig No. 190	1 c.c.	1 c.c.	1 c.c.	O.T.	○	○	8 x 8	5 x 5	10 x 10	5 x 5	15 x 15	15 x 15		
				Residue	○	○	5 x 5	○	10 x 10	8 x 8	15 x 15	15 x 15		
Guinea-pig No. 191	1 c.c.	1 c.c.	1 c.c.	O.T.	○	○	15 x 18	10 x 10	20 x 20	5 x 5	15 x 15	15 x 15	10 x 10	8 x 8
				Residue	○	○	15 x 15	5 x 5	20 x 20	10 x 10	15 x 15	15 x 15	10 x 10	8 x 8
Guinea-pig No. 192	1 c.c.	1 c.c.	1 c.c.	O.T.	8 x 8	5 x 5	18 x 18	15 x 15	20 x 20	10 x 10	20 x 20	20 x 20		
				Residue	○	○	10 x 10	5 x 5	20 x 20	15 x 15	20 x 20	20 x 20		

of the tubercle bacillus extract, left over after the acid-precipitable and heat-coagulable substances had been removed. It was not expected that this would lead to positive results, largely because our own experiments with tubercle bacillus residues and the preliminary work of Zinsser with similar extracts from other bacteria, had so far failed to lead to any antibody formation on the part of these substances.

The materials were prepared as described in the first part of this paper.

As was expected, in spite of the injection of considerable amounts of these substances into guinea pigs and repeatedly testing them at varying periods thereafter, typical tuberculin sensitiveness never was produced by this treatment.

PRODUCTION OF ANTIBODIES

In the preceding study the ability of the tuberculo-nucleoprotein and residue antigen to elicit the skin reaction in tuberculous animals was established, thus confirming Zinsser's previous observations. As these substances are antigens in one sense, naturally the next step was to determine their true antigenic properties, that is, their capability in stimulating the production of humoral antibodies like precipitins and complement-fixing antibodies, when homologous substances were used as antigens for these reactions.

Animals: Rabbits, guinea pigs and sheep were treated with tuberculo-nucleoprotein, residue antigen, living and killed human tubercle bacilli, and living bovine tubercle bacilli.

1. Tuberculo-nucleoprotein and

2. The residue antigens were prepared as usual. The method of preparation has already been described in detail.

3. The preparation of the dead tubercle bacilli suspension has also been described elsewhere.

4. Suspensions of living tubercle bacilli were prepared as follows: Young four-week-cultures, either of human H 37 or bovine B 1 origin, were triturated in a mortar with 0.85 per cent sodium chloride solution. This was allowed to stand for $\frac{1}{2}$ hour in a dark place. At the end of this time, generally, the clumps had settled to the bottom of the tube. The supernatant fluid was diluted so that it was slightly opalescent. We admit that this method is crude, but from former experiments it would seem that the method would be sufficiently accurate for our present purposes. The injections were made either subcutaneously or intraperitoneally.

The injections of tuberculo-nucleoprotein and residue antigens were done intraperitoneally, with the exception of a few which were made subcutaneously.

After allowing what we considered a suitable period to elapse, precipitin and complement-fixation tests were done with the sera of the treated animals and repeated at definite intervals over considerable periods.

Complement fixation was performed with sera which had been inactivated at 56° C. for ½ hour. The hemolytic system was a rabbit-antisheep one, and two units of amboceptor were used in the test. Ten per cent guinea pig complement and 5 per cent sheep red cells were used. In the first few series only the tuberculo-nucleoprotein and residue antigen were used as antigens, dissolved in 20 cc. of alkaline salt solution, which was further diluted 1 to 50 with 0.85 per cent salt solution. One-tenth cubic-centimetre of this final dilution was our unit in the test. The "residue" substance was always used in the undiluted state with 0.1 cc. as the unit.

The primary incubation, that is, of the antibodies, antigen and complement, was 1½ hours at 37.5° C. in a water bath. The secondary incubation after the addition of the hemolytic system was long enough to completely hemolyze serum and antigen-serum and antigen control, which was in double quantity in both. The readings were made two hours after the hemolysis had taken place in the control tubes.

The precipitin tests were done with active sera. The antigens used were the same as those used in the complement-fixation test. Test-tubes of small dimensions, and the ring test were carried throughout.

Series I: Ten rabbits of different weights were injected, three of which died early in the course of immunization, leaving 7 to be reported.

A: *Injections of Nucleoproteins:* Rabbits A277 and 278 were injected on January 12, 1922, with 2 cc. of tuberculo-nucleoprotein; on January 14, with 4 cc.; and on January 17, February 3, 8, 17, 24, March 15, 23, 25 and April 6, 1922, with 5 cc. of tuberculo-nucleoprotein, a total of 51 cc. of this material.

B: *Residue:* Rabbit A279 was injected intraperitoneally with 5 cc. of "residue" substance, on January 12, 14, 17 and February 3, a total of 20 cc. in four injections.

Rabbit A280 was injected intraperitoneally on January 12, 14, 17 and February 3, 8, 17 and 24, March 15 and 23 and April 6 with 5 cc. of "residue," a total of 50 cc. in 10 injections.

C: *Killed Tubercle Bacilli:* Rabbit A170 was injected three times intraperitoneally with 1 cc. of tubercle bacilli killed at 80° C. for one hour, a total of 3 cc.

D: *Living Tubercle Bacilli:* Rabbits A171 and A172 were injected, the former with 2.5 cc., divided into three injections, and the latter with 2 cc. in two injections. The first injection was subcutaneous and the others intraperitoneal. The strain of human bacilli was H 37.

January 21, 1922: Fourteen days after the beginning of the experiment, and nine days since the last injection, complement fixation tests were begun with the sera of the rabbits and with that of a normal rabbit as control. The results of these tests are tabulated in table 5.

Precipitin reactions were also repeatedly carried out with the sera of these rabbits. Since the results in this particular experiment are easily described we omit charting the precipitation results. Positive precipitin reactions were obtained when the sera of the "nucleoprotein" animals were allowed to react with the "nucleoprotein" antigen. These sera, however, did not precipitate with the residue antigen. The sera of the rabbits injected with residue antigens and with dead and living tubercle bacilli gave no precipitin reactions with either of the antigens employed.

Summary of Experiment I: The results of this experiment may be summarized by stating that rabbits injected with nucleoprotein derived from the tubercle bacillus developed powerful complement-fixing and precipitating antibodies for homologous nucleoprotein. Such rabbits at a later date also developed complement-fixing properties with the so called "residue" antigens. The injection of "residue" antigen, at least to the extent practised in this experiment, gave rise to no determinable antibody formation. The sera of rabbits injected with dead and living tubercle bacilli gave rise to the development of positive complement-fixing bodies in the serum, but no precipitins could be found.

Series II: In this series the immunizing injections of the rabbits were intensified over those of the preceding series. Immunizations were carried out as follows:

A: *Nucleoprotein Rabbits:* Rabbit A426 received nucleoprotein as follows: May 12, 5 cc.; May 16, 5 cc.; May 20, 10 cc.; May 22, 10 cc.; June 27, 5 cc.; July 1, 5 cc.; July 6, 5 cc.; July 12, 5 cc. and July 24, 5 cc.; a total of 55 cc. Rabbit 5 received 5 cc. on June 27, July 1, 6, 12 and 24, a total of 25 cc.

B: *Residue Material:* Rabbit A429 was intensively treated with residue material. Treatment was begun by injecting 10 cc. at an injection, five injections per day, for four days, May 12, 16, 20 and 22, respectively, receiving in this period a total of 200 cc. An interval was allowed and the treatment resumed on June 27, this rabbit receiving a further 60 cc., in amounts ranging from 10 to 15 cc. at an injection, between June 27 and July 24. Rabbit 400 received only these last 60 cc. between June 27 and July 24.

C: *Killed Human Tubercle Bacilli:* Rabbit 427 received 15 cc. of a suspension of killed human tubercle bacilli between May 12 and 26. An interrup-

TABLE 5

Complement-fixation rabbits treated with tubercle bacilli and their products

Series I

Rabbit No.	Material.		Jan. 26, 1922.			Feb. 3, 1922.			Feb. 9, 1922.			Feb. 14, 1922.			Mar. 15, 1922.			April 14, 1922.			April 12, 1922.		
			.05 c.c.	.025 c.c.	.01 c.c.	.05 c.c.	.025 c.c.	.01 c.c.	.05 c.c.	.025 c.c.	.01 c.c.	.05 c.c.	.025 c.c.	.01 c.c.	.05 c.c.	.025 c.c.	.01 c.c.	.05 c.c.	.025 c.c.	.01 c.c.	.05 c.c.	.025 c.c.	.01 c.c.
A 277	Tuberculo-nucleo-protein	T.N.P.	+++	+++	++	+++	+++	+–++	+++	+++	++	+++	++	+	+++	++	+	+++	+++	++	++++	+++	+++
		Residue	++	±	O	O	O	O	O	O	O	O	O	O	O	O	O	++	+	O	+++	+–++	O
A 278	Tuberculo-nucleo-protein	T.N.P.	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	+–++	++++	+++	++	++++	+++	+++
		Residue	+	O	O	O	O	O	O	O	O	O	O	O	O	O	O	+++	++	+	+++	++	+
A 279	Residue	T.N.P.	+	±	O	O	O	O															
		Residue	O	O	O	O	O	O															
A 280	Residue	T.N.P.	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
		Residue	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
A 170	Dead Tbc., killed at 80° C.	T.N.P.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	++	+	O	++++	+++	+
		Residue	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+	O	O	+++	++	O
A 171	Living human, H^{37}	T.N.P.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+++	++	O	++++	++	+
		Residue	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+	O	O	++++	+++	O
A 172	Living human H^{37}	T.N.P.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	++	+	O			
		Residue	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	+	O	O			
Normal		T.N.P.	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
		Residue	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

tion similar to the one practised on the other rabbits was necessitated, owing to transportation of the rabbits, and on June 27, injections were resumed, and five further injections of 2 cc. each were given between June 27 and July 24. Rabbit 401 received five injections of 2 cc. of the same killed human tubercle bacilli between June 27 and July 24.

D: *Living Human Tubercle Bacilli:* Rabbits 402 and 403 were each subcutaneously injected with 1 cc. of a suspension of living tubercle bacilli of strain H 37 on June 27.

Complement-fixation tests and precipitation reactions were carried out with the sera of these rabbits between June 26 and November 11, 1922, at seven different times. The results of the complement-fixation tests are presented in table 6.

Precipitation reactions, carried out with the sera of these rabbits, are not charted because there are again only a few points of importance which can be easily described. Precipitin reactions in this series were obtained only with the sera of two rabbits, namely, rabbit 5, which had been treated with nucleoprotein, and rabbit A427, which had received injections of killed human tubercle bacilli. In both cases positive precipitations were obtained on July 19, that is, before the immunization injections had been completed. The serum of nucleoprotein rabbit 5 precipitated at this time only the nucleoprotein antigen; whereas, strangely enough, the serum of rabbit 427, treated with killed human tubercle bacilli, at this time precipitated the residue substance, but not the nucleoprotein. Later, by August 11, both of these rabbits gave precipitation reactions with both of the antigens, and continued to give such reactions up to the last test on November 11. The sera of all the other rabbits were, like the normal controls, negative throughout; and, since they were always done at the same time, they sufficiently control the possibility of false reactions having been obtained.

Series III. In this experiment some of the rabbits used in series II were further immunized and new rabbits were added as follows:

A: *Nucleoprotein:* Old nucleoprotein rabbit 5 was further injected with a total of 21 cc. of nucleoprotein, between January 25 and February 17. New rabbit 4 received similar injections on the same days.

B: *Residue Antigens:* Old rabbit 400 was further injected with 50 cc. of the residue antigen, between January 25 and February 17. This rabbit had thus received 110 cc. of this material between January 27 and February 17. New residue rabbit 166 received 5 injections, totaling 50 cc., between January 25 and February 17.

TABLE 6

Complement-fixation rabbits treated with tubercle bacilli and their products

Series II

Rabbit No.	Material.	June 26, 1922.		July 13, 1922.		July 19, 1922.		July 27, 1922.		Aug. 11, 1922.		Sept. 2, 1922.		Nov. 11, 1922.	
		T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.
A 426	Tuberculo-nucleo-protein	+	++	++	++	+++ – ++	+	++	++	++	+++	+++	+++	++	++
5	Tuberculo-nucleo-protein	O	O	++	+	+++	+	+++	+	++++	++	++++	++	++++	+++
A 429	Residue	O	O	O	O	O	O	O	O	O	O	O	O	O	O
400	Residue	O	O	+?	O	+	+	+	+	O	+	O	O	O	O
A 427	Killed human; 80° C.; one hour	O	O	++	+	+++	++	++++	+++	++++	+++	++++	+++	++++	+++
401	Killed human; 80° C.; one hour	O	O	+	O	++	++?	++	++	+++	+++	++++	+++	++++	+++
402	Living human, H[37]	O	O	+?	O	+	+	O	O	++	++	++	+	O	O
403	Living human, H[37]	+?	O	O	O	O	O	O	O	O	O	O	O	O	O
7	Normal	..	..	O	O	O	O	O	O	O	O	O	O	O	O
8	Normal	..	..	O	O	O	O	O	O	O	O	O	O	O	O

C: *Killed Human Tubercle Bacilli:* Rabbits 28 and 106 received 2 cc. of killed human tubercle bacilli, killed at 100° C. for one hour on January 25.

D: *Living Human Tubercle Bacilli:* The two rabbits, 402 and 403, used in the previous series, being in good condition, received a second injection of 2 cc. of an emulsion of living bacilli of human strain H 37.

E: Another factor was brought into this experiment by the addition of two rabbits 525 and 140, each of which received a suspension of 2 cc. of living bovine tubercle bacilli B 1.

Complement-fixation reactions were carried out with the sera of these rabbits, and the two antigens used in previous experiments as indicated in table 7.

Precipitin reactions with the sera of the rabbits of this series were similar to those in the experiments reported above. The rabbits treated with nucleoprotein eventually gave precipitation reactions with both antigens. Rabbit 402, treated with living human bacilli, developed moderate precipitin reactions for both antigens by April 2; and rabbit 28, treated with killed human tubercle bacilli, developed similar precipitins between February 10 and April 2. An interesting feature of this experiment is that the rabbits injected with living bovine bacilli developed neither complement fixation nor precipitation for any of the antigens used. The observations in this regard are too few to lead to any definite conclusions, but the observation is sufficiently interesting to be mentioned in passing.

SUMMARY OF EXPERIMENTS OF ANTIBODY FORMATION IN RABBITS

We may summarize the experiments on the immunization of rabbits with nucleoproteins, residue antigens, dead and living human tubercle bacilli, as follows:

Sufficient immunization with the nucleoprotein fraction of tubercle bacillus extracts leads to the development of complement-fixing and precipitating properties in the sera, for both the nucleoprotein and the so called "residue" antigen.

Treatment with dead and with living human tubercle bacilli in some cases also develops complement-fixing and precipitating properties for both antigens.

In no case did treatment with the residue antigens lead to any evidence of antibody formation whatever. This observation is so far consistent with the observations made in the case of other bacteria, by Zinsser and

TABLE 7

Complement-fixation rabbits treated with tubercle bacilli and their products

Series III

Rabbit No.	Material.	Jan. 26, 1923.		Feb. 9, 1923.		Feb. 20, 1923.		Mar. 8, 1923.		April 2, 1923.	
		T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.	T.N.P.	Residue.
5	Tuberculo-nucleo-protein	++	O	++++	+++	++++	+++	++++	++++	++++	++++
4	Tuberculo-nucleo-protein	O	O	+++	O	+++	O	++++	++	++++	+++
166	Residue	O	O	O	O	O	O	O	O		
400	Residue	O	O	O	O	O	O	O	O	O	O
403	Living human, H[37]	O	O	O	O	O	O				
402	Living human, H[37]	O	O	+++	++	+++	++	+++	+++	++++	+++
525	Living bovine, B	O	O	O	O	O	O	O	O		
140	Living bovine, B	O	O	O	O	O	O				
28	Killed human; 100° C.; one hour	O	O	O	O	++	O	+++	+		
106	Killed human; 100° C.; one hour	O	O	O	O	++	O	+++	+	+++	++
Normal		..	..	O	O	O	O	O	O	O	O
Normal		..	..	O	O	O	O	O	O	O	O

his associates. Although in both laboratories further work on this problem must be done, with a more intensive attempt to produce antibodies with these substances, it appears at present as though this residue substance possessed the capacity of union with antibodies, without possessing the true antigenic property of inducing antibody formation after injection into animals.

THE DEVELOPMENT OF ANTIBODIES IN GUINEA PIGS TREATED BY VARIOUS METHODS

Since, in the course of our other experiments on skin reactions, we had treated a considerable number of guinea pigs with tubercle bacilli and their various derivatives, we thought it interesting to determine, if possible, whether or not antibodies appear in the course of such treatment. The guinea pigs had been variously treated with tubercle bacilli killed at 100°C., with filtered and unfiltered nucleoprotein, and infected with living tubercle bacilli.

We may briefly summarize the results as follows:

The most powerful complement fixations were obtained with animals treated with dead tubercle bacilli, and with the unfiltered nucleoprotein preparations. Complement fixations were also obtained, but more feebly and more irregularly, in the animals treated with filtered nucleoproteins and in the tuberculous animals.

No reactions of any kind were ever obtained in animals treated with residue antigens.

The most noticeable feature of these reactions is the fact that, while the tuberculous animals developed the most powerful skin reactions, these animals developed the feeblest and most irregular complement-fixation reactions. The animals treated with killed tubercle bacilli, as described above, also developed typical skin reactions; but, in addition to this, they developed powerful complement-fixation reactions, which were absent in the animals infected with the living bacilli. This point seems to us of great importance; but, we refrain for the present from drawing any theoretical conclusions, since more work must be done on the subject before this is possible.

EXPERIENCE WITH SHEEP

When we began our experiments we had hoped to be able to do a considerable amount of work with the sera of animals immunized with

the different fractions. We attempted, therefore, to immunize sheep with the nucleoprotein fraction derived from tubercle bacillus extracts, injecting considerable amounts. Unfortunately, it was found that after the first few injections the sheep became highly susceptible to the nucleoprotein injections, and three sheep were lost in this way. There was no other explanation for the death of the sheep, except the acquisition of a powerful hypersensitiveness to the injected material. After this, we were forced to abandon such treatment and to produce our tuberculous antisera by the older methods of injecting dead and living bacilli.

Sheep so treated rapidly develop complement-fixing and precipitating antibodies after four or more injections.

GENERAL SUMMARY

The work above recorded concerns itself with the reaction of the animal body to injections with substances extracted from pulverized tubercle bacilli, and a comparison of these reactions with those induced by the injection of dead tubercle bacilli and by infection with living bacilli.

The work is a direct continuation of a general plan for the investigation of immunity in tuberculosis, begun in the bacteriological laboratory of Columbia University, and represents the beginning of a series of similar studies which the writer, in conjunction with others who have been interested in this work, intends to continue. It is, therefore, not our intention to present it as a finished study, but rather as a determination of certain preliminary facts which we have deemed necessary as a foundation for the more difficult problems presented by tuberculous infections.

We believe that our work, so far, has demonstrated the following facts:

Manifestations of skin hypersensitiveness may be elicited in animals infected with living tubercle bacilli, not only with old tuberculin and the tuberculins hitherto in general use, but also with a fraction of powdered tubercle bacillus extracts, which is precipitable by acetic acid in the cold and which we speak of in the paper as the "nucleoprotein" fraction. It may also be elicited by the "residue" antigens, described by Zinsser, from which all "nucleoproteins" and coagulable proteins have been removed. The chemical constitution of these last substances is, as yet, obscure, but is being studied at the present time in various places. The skin reactions produced with these so called "residue" materials are qualitatively identical with that produced with old tuberculin and "nucleoprotein," but they are sometimes a little weaker.

When guinea pigs are systematically treated with considerable quantities of the so called "nucleoprotein" fraction of the extracts, skin hypersensitiveness of a mild type may be elicited in many of the animals. Such skin hypersensitiveness, however, cannot definitely be claimed to be identical with tuberculin hypersensitiveness. The reactions are usually less marked than similar ones produced with the same materials in tuberculous guinea pigs; they fade more rapidly; and they never lead to deep injury of the tissues. It will remain uncertain, until further studies have been carried out, whether these reactions are a specific nucleoprotein hypersensitiveness that has no immunological relationship to the skin reactions observed in tuberculous animals, or whether they represent a milder form of the typical tuberculin reaction. Moreover, in view of our experiments on sensitization with dead tubercle bacilli, we hesitate, for the present, completely to exclude the possibility that the reactions obtained with the "nucleoprotein" injections may have been due to minute amounts of unextracted fragments of tubercle bacilli which may have passed through the filters. Although we have taken every precaution to exclude this possibility, we admit it has not been completely removed.

Our experiments have definitely shown, in confirmation of Baldwin's early experiments, that skin hypersensitiveness may be produced by the injection of dead tubercle bacilli, as well as by infection with living bacilli. Such reactions are typical, as extensive as in the infected animals, and persist as long as does the mass formed about the point of injection. Most surprising is the fact that this sensitizing effect of dead tubercle bacilli is not removed by heating as high as 121°C. under 15 pounds steam pressure. These facts suggest many speculations concerning the nature of the reactions which incite skin hypersensitiveness. But any expression of opinion regarding this must be postponed until the studies which obviously suggest themselves have been undertaken.

The so called "residue" materials injected in considerable quantities have never elicited any kind of skin hypersensitiveness to any of the various preparations used.

Our antibody studies have shown that complement-fixing and precipitating antibodies may be produced in rabbits by the injection of dead tubercle bacilli, living bacilli and the "nucleoprotein" fraction described above. In no case have the "residue" materials induced any kind of demonstrable antibody formation. This is in keeping with similar observations by Zinsser, but in both laboratories further attempts are

being made to confirm this point by more and more intensive injection of rabbits with the "residue" materials.

In spite of the inability of the "residue" materials, therefore, to induce antibody formation, these substances are nevertheless capable of inciting specific skin reactions and of giving complement fixation and precipitation, *in vitro*, with potent antisera.

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EXPLANATION OF PLATES

Plate 1

FIG. 1. GUINEA PIG A 106, SHOWING SKIN REACTIONS 24 HOURS AFTER

i: old tuberculin, 5 per cent.
ii: nucleo protein.
iii: residue substance.

FIG. 2. GUINEA PIG A 527, SHOWING SKIN REACTIONS AFTER SAME TESTS AS A 106

FIG. 3. GUINEA PIG A 528, SHOWING SKIN REACTIONS 24 HOURS AFTER

i: old tuberculin, 5 per cent.
ii: nucleoprotein.
iii: residue substance.

(Forty-two days after beginning of experiment.)

Plate 2

FIG. 4: GUINEA PIG 115, SHOWING SKIN REACTIONS 48 HOURS AFTER

i: old tuberculin, 5 per cent.
ii: residue substance.

(Thirty-two days after beginning of experiment.)

FIG. 5: GUINEA PIG 117, SHOWING SKIN REACTIONS 24 HOURS AFTER

i: old tuberculin, 5 per cent.
ii: residue substance.

(Seventy-nine days after beginning of experiment.)

FIG. 6: GUINEA PIG 191, SHOWING SKIN REACTIONS 24 HOURS AFTER

i: old tuberculin, 5 per cent.
ii: residue substance.

(Twenty-five days after beginning of experiment.)

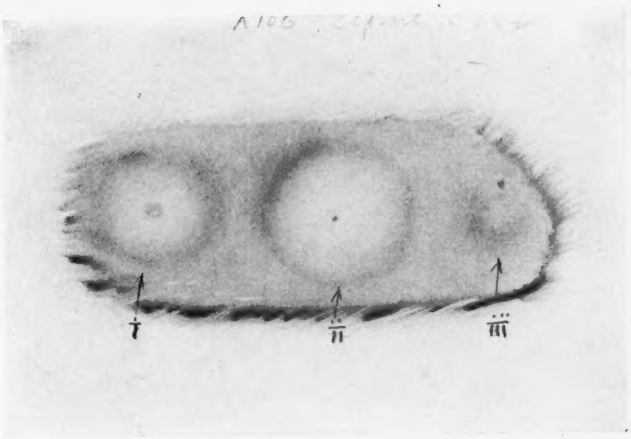


FIG. 1

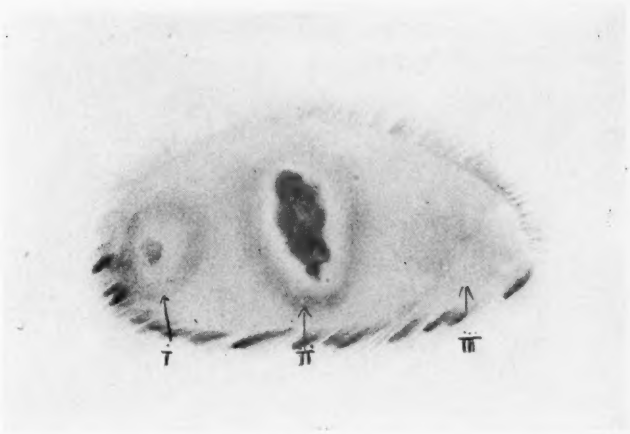


FIG. 2

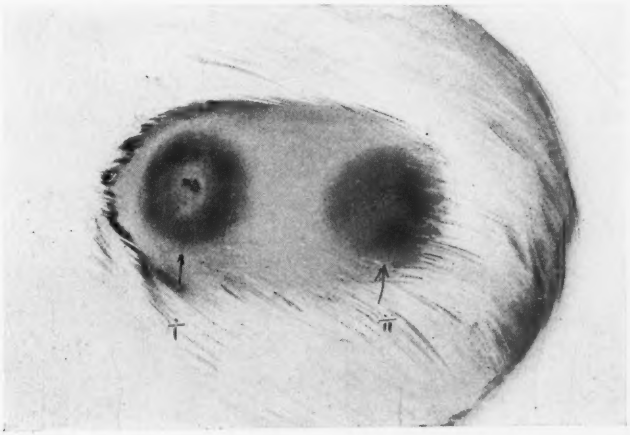


FIG. 3

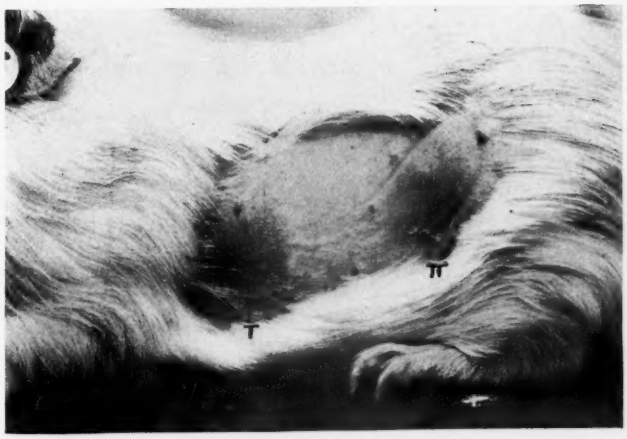


FIG. 4

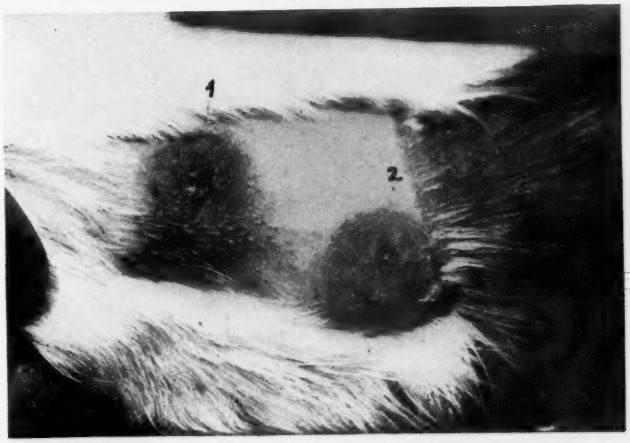


FIG. 5

FIG. 6

A STATISTICAL STUDY OF TUBERCULOSIS MORTALITY IN COLORADO FOR THE THIRTEEN YEARS 1908 TO 1920[1]

The following report had its inception at a meeting of the Denver Sanatorium Association, held October 26, 1920, at which a committee, consisting of Dr. Spivak, Dr. Holden and Dr. Beggs, was appointed to investigate the relations of climate, and in particular that of Colorado, to the development of tuberculosis. The committee was empowered to increase its membership and was finally constituted as follows:

W. N. Beggs, M.D.	Henry Sewall, M.D., *Chairman*
R. L. Drinkwater, M.D.	C. D. Spivak, M.D., *Secretary*
G. W. Holden, M.D.	A. S. Taussig, M.D.

During the past generation general scientific and clinical opinion has drifted from the view that climate is the paramount environmental factor, both in the development and treatment of tuberculosis, to the postulate that climate, *per se*, plays little or no part either as a restraining or curative medium against tuberculosis.

The mortality rate from tuberculosis in Colorado, as set forth in the *U. S. Census Mortality Statistics*, is represented as higher than that of any other state save one. This extraordinary statement may indicate either that Colorado presents conditions which exceptionally favor the inception and development of tuberculosis or, on the other hand, that the climate of the State offers so salubrious an environment as to attract from everywhere a horde of invalids who come hither in vain effort to reëstablish health. The judicial observer must suspect equally the validity of statements which, on the one hand, incriminate Colorado with especially breeding tuberculosis and, on the other, announce the opinion that mortality from tuberculosis developed in Colorado is practically unknown.

The committee, whose report follows, realized that the only hope of securing reliable information on the intricate problem set for it was

[1] Conducted by a Committee of the Denver Sanatorium Association, under a grant from the National Research Council and with the coöperation of the Colorado State Board of Health.

through an attack directed on the simplest group of conditions which analysis of the problem could disclose.

It was accordingly determined to compile the data of tuberculosis mortality in Colorado for ten years, ending with 1920, as represented on the death certificates filed with the Secretary of the Colorado State Board of Health. *The object of the investigation was to determine the ratio of deaths from tuberculosis developed in Colorado to the whole number of deaths from tuberculosis in the State.*

We have avoided the obscure term "disease contracted." Data regarding the implantation of tubercle bacilli or of the presence of latent tuberculosis are wholly lacking. The disease was assumed to have originated in that locality in which first appeared the signs and symptoms leading to the clinical diagnosis of tuberculosis. We have assumed that the word "contracted" in the death certificate means "developed."

An important use of death certificates is to throw light upon living conditions whereby they may be ameliorated. Much in the same way as the ratio of gas exchange which expresses the basal metabolism of an individual is the resultant of innumerable unknown vital reactions, so the indisputable resultant, *death*, is determined by a great variety of antecedent environmental conditions which had affected the decedent and ordered his fate. At least this is the point of view of the interested amateurs who have prosecuted this research.

Brief experience with the enormous mass of material to be reviewed made it plain that our project could only be carried through with considerable outlay of time and expense. By good fortune Dr. V. C. Vaughan, then Chairman of the Medical Division of the National Research Council, became interested in the problem, and through his offices a grant of $1000 was made by the National Research Council for the salary of assistants. Even so, the work would have been impossible without the generous donations of office room and printing by the Colorado State Board of Health through its Secretary, first, Dr. R. L. Drinkwater, and later, his successor, Dr. Tracy R. Love.

It will be perceived that the foundation for any conclusions to be deduced from this compilation must have been furnished by upwards of 1700 clinicians practising in Colorado. They were the final arbiters of our classification of facts. Varied as they were in character and education, subject to errors in diagnosis and imposed with the task of tracing the social history of their patients, it is to be supposed that a

considerable element of error enters into the fundamental material which we had to assemble. Nevertheless, as the work progressed it gave internal evidence of essential accuracy.

Our chief task, therefore, was to scrutinize, clarify and verify the death certificates reporting tuberculosis mortalities. This work was supervised by a member of the committee, but was directly conducted by Miss Eunice M. Hopkin, whose conscientious, intelligent and accurate service is worthy of every praise. Her recent untimely death gives hope that this paper may form a worthy monument to her fidelity to duty.

The preliminary task consisted in culling 23,608 certificates of deaths attributed to tuberculosis from a much larger mass of material, and in critically classifying the data contained therein. In compiling the data determining the mortality from indigenous tuberculosis in Colorado many side lines of vital interest developed, so that the original scope of our investigation became greatly amplified.

When a returned death certificate contained definite answers to the printed questions it was accepted and its appropriate data were entered on our record sheet. When, however, there was ambiguity or important omission in the replies a letter was directed to the physician at fault, of which the following is a copy:

COMMITTEE ON THE INVESTIGATION OF TUBERCULOSIS IN COLORADO

OFFICE OF THE COLORADO STATE BOARD OF HEALTH

Capitol Building, Denver

Henry Sewall, M. D., *Chairman,*	W. N. Beggs, M. D.
C. D. Spivak, M. D., *Secretary*	A. S. Taussig, M. D.
G. W. Holden, M. D.	R. L. Drinkwater, M. D.

Dear Doctor:

The above named Committee has been commissioned by the National Research Council of Washington, D. C., and approved by the Governor of Colorado, to determine, if possible, the status of tuberculosis mortality in Colorado.

This Committee is working in coöperation with the Colorado State Board of Health.

We find many death certificates incomplete. For this reason we are asking you to coöperate with us in obtaining the necessary data to complete the files of this office.

May we ask you to supply the needed information from your records, on the enclosed postal card, concerning the data contained on the State certificate No....., Year ....., and signed with your name in regard to the death of M. ..... of ....., date ..... The cause of death was reported as.....

If it is impossible for you to give the required information, please so state under "Remarks" and return the postal card at the earliest possible moment.

With appreciation of your attention to this matter,

We remain, very truly yours,

(Signed) TRACY R. LOVE, M. D.,
Secretary State Board of Health.

The enclosed postal card was printed with the following questionnaire:

State Certificate No ....., Year .....

Please answer questions marked with (X)

( ) Length of residence in Colorado: Yrs....., Mos.....
( ) Duration of disease: Yrs....., Mos.....
( ) Where was Tb. contracted? In Colo.? (Yes) (No)
( ) Was Tb. the direct cause of death? (Yes) (No)
( ) Was Tb. contributory cause of death? (Yes) (No)
( ) Was there evidence of Pulmonary Tb.?
(Reported as Miners' Consumption)? (Yes) (No)

Remarks.....
.....

Signed.....M. D.

If a letter failed to receive a reply a second one was usually sent. The proportion of replies satisfactorily correcting omissions in the death certificates was noteworthy. The average of satisfactory replies for thirteen years was over 49 per cent of the inquiries addressed. The percentage varied from 33 for the mortality of 1908 to 65 in 1919. The yearly number of letters of inquiry averaged 395. It varied between 115 for 1908 and 1027 in 1919.

It is self evident that the correspondence with individual physicians must have become progressively less voluminous with receding years, due to death or migration of the practitioners. But there was a notable increase in the proportion of certificates lacking certain data beginning with the year 1918. In this year the form of the U. S. standard death certificate was revised. In the old certificate all the data regarding a decedent which the physician was required to furnish were grouped

together on the right hand side of the blank. Prominence was given queries as to *Length of residence*, first, at the place of death, and second, in the State. In the new certificate the second question, for our purpose of prime importance, was abandoned altogether and the first was transferred to an inconspicuous place on the left of the blank and must have been answered by another than the medical attendant. The new certificates first began to appear in the Colorado files in September, 1918, and by the middle of 1919 they had completely supplanted the old form.

It may be said here that internal evidence, from data to be considered later, indicated that no material inaccuracy involved the returns as to the length of sojourn of decedents in Colorado, due to adoption of the new form of certificate during the last two years of our compilation.

A noteworthy fact, for which no explanation is obvious, is a sudden increase in the proportion of fully satisfactory certificates which developed between 1913 and 1914. For the six years 1908 to 1913 the average percentage of satisfactory corrections by correspondence of deficient death certificates was about 40.9; and the average ratio of completely satisfactory certificates to the whole number of deaths from tuberculosis was 56.5 per cent. For the seven years 1914 to 1920 these averages increased to 75.4 and 85.3 per cent, respectively. Inspection of chart 1 shows graphically the relative excess of deaths in cases of "unknown" origin occurring before 1914. The fundamental aim of our investigation was to segregate from each other the deaths from tuberculosis according to the locality in which the disease was developed, whether in or out of Colorado.

It is obvious that a physician is often qualified to render a reliable decision as to *where* the decedent resided at the time his disease developed when he would be quite unable to satisfy completely the somewhat elaborate questionnaire of the death certificate.

In our collation of tuberculosis statistics the data were transferred directly from the filed death certificates to printed sheets, measuring 14 by 26 inches, ruled and divided into columns with the following captions: (*1*) *No.; Place of Death; Length of Residence in Colorado; Sex, Race or Color; Single, Married, Widowed or Divorced; Age; Occupation; Birthplace; Date of Death; Form of Disease; Duration of Disease; Where Developed;* (*2*) *Colorado Number; Name of Deceased; Attending Physician and Address; Condition of Exposure; Certificate Number; Date of Letter Sent; Date of Reply; Remarks.*

It will be observed that the second half of this questionnaire refers wholly to deaths in cases in which disease was alleged to have been developed in Colorado, and the chief burden of the compilation was involved in the sifting of the evidence pertaining to this group of cases.

The record sheets described were grouped in time units of one month, and their data were transferred to special multigraphed blanks, each of which accommodated the statistics of one month or contained the summary for a whole year. These blanks were the direct bases of all calculations. They measured approximately 11 by 13 inches. The first column on the left segregated, from above downward, the deaths according to the place where the disease originated, as *Out of Colorado*, *In Colorado*, *Unknown*. The second column segregated the various forms of tuberculosis in four groups, *Pulmonary*, *Miners' Consumption*, *Meningitis* and *Other Forms*. This classification was repeated for each of the three possible places of disease development. The remainder of the blank was chiefly occupied with the numerical death records distinguished as to sex and distributed according to age as follows: *0–2; 3–9; 10–19; 20–29; 30–39; 40–49; 50–59; 60 and over*. Columns for totals, according to sex, bounded the foregoing aggregate on the right and below. The lower half of the sheet was devoted to enumeration of the diseases occurring as complications, also classified according to origin of the cases of tuberculosis in which they occurred. Finally, at the bottom of the sheet provision was made for the representation of grand totals, and the number of complete and incomplete certificates. Table 6 is reproduced from one of the blanks which had been filed.

In setting forth the data accumulated it was obviously inexpedient to transcribe the records as assembled. The effort was made to fix upon the salient facts to be determined and to group them for the whole period covered by our investigation upon a single sheet, as has been done in table 1.

THE SUMMARY OF TUBERCULOSIS DATA

In table 1 the principal data of the tuberculosis mortality in Colorado for thirteen years are set down in adjoining columns. The table represents a total mortality from tuberculosis in Colorado for thirteen years of 23,608 deaths, of which 20,564 involved cases in which the place of development of the disease is known. The letters *M* and *F* under the year number represent, of course, the number of males and females making up the total, in heavy ink. A special line is devoted to recording

cases of known origin, representing the sum of cases developed outside Colorado and those developed within the State.

It will be observed that deaths in cases developed in Colorado form from 12.9 to 20.1 per cent of all deaths in cases of known place of origin, the average for thirteen years being 16.9 per cent.

As our investigation was confessedly an inquiry into medical climatology, it was thought important also to represent the effect upon the mortality percentage of deaths in cases developed in Colorado of subtracting from the Colorado mortality the number of deaths due to conditions obviously unconcerned with climate. Two outstanding causes of death in Colorado come within this category: one is *Miners' Consumption*, and the other, *Primary Meningitis*, in the first decade of life. It will be observed that the effect of this subtraction diminishes by about three units the percentage of indigenous Colorado mortality.

In transcribing the mortality records care was taken to note in brackets such tuberculous complications as might be added to the original or main cause of death. Thus (table 6) the cause of death might be given as *Pulmonary Tuberculosis;* the secondary or complicating cause as *Tuberculous Meningitis*. In this event the death was recorded as from pulmonary tuberculosis while, in the same column, opposite the caption *Meningitis* a numeral in brackets, (*1*), designated the occurrence of the complication. The bracketed numbers were not, of course, included in the mortality rates. Now, a curious and constant result was noted after the compilation of these figures; namely, that the proportion of tuberculous complications in cases originating in Colorado to all indigenous cases was notably less than the proportion of tuberculous complications to cases of the disease originating outside Colorado. These percentage relations are represented in round numbers and are set down just above the heavy figures denoting the actual number of cases with tuberculous complications. The average percentage of deaths for thirteen years in cases developed in Colorado presenting tuberculous complications was about 4.6; in deaths in cases developed outside Colorado it was about 9.4.

At the bottom of chart 4 is represented graphically the average percentage of deaths from multiple tuberculous lesions in cases developed outside and in Colorado, respectively.

It is easily conceived that the medical practitioner who seeks to record fully and truly the cause of death in a case of tuberculosis is often in quandary as to the relative importance to be attached to the

tuberculous process and to the frequently associated, often acute, nontuberculous disease. The disturbance in the mortality rates of tuberculosis, incident to the influenza epidemic and involving the years 1918 to 1920, was manifested by such an abnormal obtrusion of fatalities from acute nontuberculous pulmonary disease that we were advised by high authority to reject the statistics of those years; but we decided to add them to our decade of investigation for the sake of comparison. The filed death certificates note a variety of some fifty different nontuberculous diseases which were associated with the tuberculosis and more or less complicated it.

These associated diseases were numerically unimportant, with the constant exception of four, namely, influenza, inseparable from influenza-pneumonia, nephritis and cardiac disease. We must accept the general characterization of these groups, without insisting on accurate pathological classification of each. Each column of table 1 gives the actual number of deaths in cases with either of the principal associated diseases; also, in round numbers, the percentage of the total tuberculosis mortality formed by the cases with the associated disease. In view of ordinary clinical teaching the high rate of complicating cardiac disease is noteworthy. The sudden increase of fatalities beginning in 1917 and continuing through 1920 will be discussed later.

In the lower half of table 1 the mortality statistics are assembled with reference to the *Form of the Disease.* The deaths are numerically segregated according to the locality in which the tuberculous disease developed; whether *out* of Colorado (*O*), *in* Colorado (*I*), or in places unknown (*U*). The outstanding cause of death is, of course, pulmonary tuberculosis.

It has been thought wise to note specifically the incidence of *Miners' Consumption,* which forms so considerable a proportion of deaths in indigenous cases of tuberculosis. It is well known that, in a great proportion of cases, mineral deposits of certain kinds in the lungs sooner or later lead to the development of a fatal form of pulmonary tuberculosis. In our review of mortality data special care was taken not to include in our records deaths due to miners' consumption, pure and simple, but only those in which the disease tuberculosis closed the history. Correspondence with local practitioners usually gave satisfactory classification in this matter. This topic will be treated later.

GRAPHIC REPRESENTATION OF TUBERCULOSIS IN COLORADO ACCORDING TO PLACE OF DEVELOPMENT

Tracings representing the mortality from tuberculosis in Colorado according to geographical origin of the disease are given in charts 1 to 4. Favorable commentary on the increasing fund of reliable data is furnished by the marked descent of the curve of *Unknown Origin*, the solid line at the bottom of chart 1, after 1911.

It will be observed (chart 1) that there is a nearly steady decline in the total number of deaths from tuberculosis from 1908 to the end of 1916. This decline is, for the most part, not shared by the curves representing cases of known origin, for these are continually increased by additions from the class of "unknowns." This chart must be viewed in connection with chart 2, in which the Colorado mortality per 100,000 population is compared with that throughout the Registration Area. Chart 1 shows an extraordinary augmentation in the mortality ascribed to tuberculosis from 1917 to 1920 inclusive. This was the period of the first epidemic of influenza. The rise in mortality involves chiefly the group of tuberculosis cases in which disease developed outside Colorado, and is shared to only a very limited extent by cases of tuberculosis indigenous to the State. The same may be said of the tuberculosis throughout the Registration Area. The explanation of this excessive mortality among tuberculous immigrants to Colorado is, as will be advanced later, probably to be found in the fact that so large a proportion of them delay their advent until the disease is far advanced, rendering them incapable of resisting the additional infection with influenza.

TUBERCULOUS MENINGITIS

There is no intention here of entering into debatable questions of pathology. How frequently, if ever, an initial infection with tubercle bacilli involves the meninges is of purely academic consideration.

According to the form of death certificate now in use, the reporting physician is required to state the *Cause of Death* and to give the duration of the disease; then follows space for notation of the *contributory* or *secondary* disease with its duration. The medical attendant who observes an apparently healthy infant develop characteristic nerve-muscle symptoms and succumb within a comparatively brief period inevitably assigns "meningitis" as the cause of death. Under certain

conditions of history and environment he further ventures to designate the etiology as "tuberculous."

We call this *Primary Tuberculous Meningitis.* When, on the other hand, the same medical attendant sees a patient with chronic tuberculosis of any organ fall ill and later develop meningeal symptoms and die with these, he is very apt to set this down as "contributory or secondary" tuberculous meningitis.

Our records show that this point of view leads to a natural classification of disease. The most striking feature of our statistics is the high incidence of primary meningitis in the comparatively small group of cases in which the disease was developed in Colorado. This is due, of course, to the fact that practically all of the relatively high infantile tuberculosis mortality belongs to our group of indigenous tuberculosis.

It must be remembered that "primary" tuberculous meningitis is distinctively a disease of infancy. According to the *Mortality Statistics of the Census* the "median age" of death in tuberculous meningitis throughout the Registration Area is about two years. That is, just as many deaths from disease occur before the age of two as are due to it in all succeeding years. The *Mortality Reports of the Census* point out the wide difference between the "median age" and the "average age" at death. Table 1 does not portray the incidence of disease according to age, but on chart 5 is represented graphically the average age of death from meningitis for thirteen years. It will be seen that the highest mortality from primary meningitis occurs within the first two years of life; then follow a rapid decline until the tenth year, a slower decline until the twentieth year, and finally a very gradual diminution in mortality that is somewhat accelerated in the fifth decade.

It will be observed in table 1 that the number of deaths from secondary meningitis is notably large in cases developed outside Colorado (*O*), as it is small in cases developed inside the State (*I*). On chart 5 it will be seen that the secondary meningeal disease, including cases from all localities, is numerically insignificant until the twentieth year but rapidly attains a maximum by the thirtieth year. It will be noted that there is a distinct parallelism between the curve of secondary meningitis and the curves representing general mortality from tuberculosis according to age on the same chart. Quite a different course is taken by the curve representing primary meningitis during the first two years of life.

It was supposed at the outset of our investigation that the presumably relatively increased exposure to tuberculosis suffered by infants born in Colorado must lead to an excessive incidence of tuberculous meningitis in the early years of life in Colorado as compared with the whole Registration Area. The Census provides data from which was calculated the mortality rate for tuberculous meningitis during the first decade of life in the Registration Area. The same calculation was made from the vital statistics of Colorado. To our astonishment it was seen that the mortality rate for the former is about twice that for for the latter population (table 2). In this connection a little reflection very well illustrates the futility of conclusions which may be founded on correct premises.

It is demonstrable, as illustrated in table 1 and on chart 5, that an excessive proportion of the cases of infantile meningitis, dying in Colorado, developed their disease on the spot, and their deaths form a part of the mortality attributed to that group of cases defined as indigenous, or developed in Colorado. It seems proper, therefore, to consider the proportion borne by deaths from infantile tuberculosis to the whole number of deaths *originating* in Colorado.

This is represented in table 2, lines 9 and 10, where it is shown that the deaths from tuberculous meningitis in the first decade of life form from 7.7 to 14.9 per cent of all deaths from tuberculosis in cases developed in Colorado, and amount to from 1.2 to 2.5 per cent of the total mortality from tuberculosis in Colorado.

Our data on the mortality from tuberculous meningitis, according to sex and place of origin of the primary tuberculous disease, is collected in broad outline in table 4. The cases of primary and secondary meningitis are separated in the table, each according to sex. The first group is made up nearly altogether from deaths in cases under ten years of age, while the members of the group of secondary meningitis were drawn chiefly from adult life. The deaths are classified according to geographical residence of the case at the onset of the tuberculous disease, as outside Colorado, inside Colorado and unknown. The actual number of deaths is given, as is the relative percentage of deaths in males and females in each group.

It will be noted that a majority (67 per cent) of the deaths from primary meningitis are included in the relatively small collection of deaths from tuberculous disease developed in Colorado. The explanation is plain: the great majority of deaths from primary tuberculous men-

ingitis occur in infancy, and comparatively few tuberculous infants are brought into Colorado. It will be noted that there is a relatively small excess of male mortality in general cases developed in Colorado (cf. table 3, line 8).

As shown at the bottom of chart 5, secondary or complicating meningitis is an incident especially of adult life.

Inspection of table 4 shows a great preponderance of deaths in cases with secondary meningitis in the group in which the original tuberculous disorder developed outside Colorado, and that nearly 90 per cent of these complications were in males.

It should be noted that the incidence of *secondary* meningitis in cases originating in Colorado is small. But, when we view the record of mortality from secondary meningitis occurring in tuberculosis subjects who had immigrated into Colorado, we find that they compose 86.4 per cent of the whole number of cases of secondary meningitis and that nearly 82 per cent of them are males.

This subject will be reverted to in the consideration of sex influence on the disease. Graphs representing the average of all cases of meningitis for thirteen years, according to age, are shown at the bottom of chart 5.

It is obvious that the final group, *Other Forms of Tuberculosis*, at the foot of table 1, must include many diverse organic affections. It has been possible to distribute these, for the most part, according to geographical origin, but not, in this review, to treat them from a pathological standpoint. Here, as elsewhere in table 1, will be noted a distinct diminution in the reference of deaths to sources of "unknown" geographical origin after about the year 1913.

TUBERCULOSIS MORTALITY RATES IN COLORADO COMPARED WITH THOSE OF THE REGISTRATION AREA

Colorado presents wholly exceptional conditions of population and of environment, in a broad sense, as contrasted with the older sections of the country and, obviously, no adequate conception of the significance of its vital statistics can be entertained except through a comparison of its mortality rates with those of the general population, insufficient as may be the information to be gained through such data. The elementary fact that a death rate is the offspring of two factors, the number of people and the number of deaths, makes the accurate determination of these factors of paramount importance.

The Bureau of the Census bases its mortality reports on the returns of vital statistics from certain specified States and communities which can be relied upon to furnish accurate data. The region covered by these correspondents is known as the *Registration Area* and its swelling population is noted at the top of table 2. Colorado has formed a part of the Registration Area throughout the period covered by our investigation and *in our exposition of the data pertaining to the Registration Area no attempt has been made to eliminate the component pertaining to Colorado.*

Whenever possible, all figures set down in table 2 were taken directly from the *Mortality Statistics of the Census Bureau.* For the population of Colorado, however, it was necessary to make special estimates, by the arithmetical method, from 1913 to the census year 1920, and the estimates are made for July 1 of each year. The exhibits of table 2 should be self-explanatory. It is seen at the outset that Colorado is discredited with a death rate from tuberculosis which is far in excess of that of the general Registration Area, including Colorado. For the thirteen years under consideration the average annual deaths from tuberculosis per 100,000 in the Registration Area number 147.5; in Colorado, 208.9. The evil eminence given Colorado places this Commonwealth near the very top of the column of tuberculosis mortality rates in the United States. The same remark, of course, applies to pulmonary tuberculosis, the most outstanding form of the disease. This result must have its explanation in one of two conditions. Either Colorado is specifically a breeding place for tuberculosis, in which case there should be demonstrable evidence of the fecundity of the indigenous disease, or some conditions pertain to the Coloradoan environment which attract sufferers from tuberculosis from a broad world area, thus leading to an abnormal numerical preponderance of the tuberculous population. The absurdity of deducing conclusions in the field of medical climatology from such unqualified mortality rates would seem to be self-evident, yet such has been done by eminent authorities.

In the last three lines of table 2 the tuberculosis mortality rates of Colorado are analyzed in a way to elucidate the paradoxical suggestions conveyed by the bare statistics. When the deaths which occur from tuberculosis in Colorado are classified so as to separate "indigenous" from imported cases of disease it is found that the Colorado death rate is remarkably low. It is seen that, whereas the average death rate of the tuberculous in Colorado who developed their disease outside the State is 150.5 per 100,000 of the Colorado population, the same ratio for

the deaths from disease developed in Colorado averages but 30.8, in contrast with the rate of 147.5 per 100,000 in the Registration Area; and, when it is considered that tuberculous meningitis in the first ten years of life constitutes an average of 10.8 per cent of the Colorado indigenous mortality, we find, on excluding this, a death rate of Colorado-developed disease of but 27.5 per 100,000 population. A source of error in these calculations, dependent on the unknown ratio of native to imported population, will be mentioned later.

All calculations of mortality rates in Colorado are here made directly from the certificates filed with the State Board of Health based on population estimates as described.

On chart 1 will be found in graphic detail the numerical course of tuberculosis mortality in Colorado throughout the period of our investigation.

It will be noticed that the total number of deaths from tuberculosis diminished fairly continuously until the onset of the epidemic of influenza when it suddenly mounted. This increase in mortality is not deferred until the epidemic year 1918 but is already manifest in 1917. It will be observed that the influence of the epidemic on the total tuberculosis mortality is due nearly wholly to its involvement of those cases of tuberculosis which developed their tuberculous disease outside Colorado: the influenza affected very slightly the curve representing deaths in cases of indigenous tuberculosis (chart 1), and the same is true for the Registration Area (chart 2). It will be noticed that the component of the curve of total mortality formed by deaths in cases of unknown origin greatly modifies the curve of the former. It would appear that most of the "unknown" class belong to imported cases of disease.

In chart 2 the mortality rates from tuberculosis in Colorado and in the Registration Area are compared. It will be seen that there is a tendency to parallelism in the two sets of curves, but the variations in the curves for Colorado are much more pronounced. Two explanations are apparent for the eccentricities of Colorado mortality rates. First, what amounts almost to a propaganda against climate in the treatment of tuberculosis has been general in the past twenty years. This has probably notably reduced the immigration of the tuberculous invalids to Colorado. Therefore, there is a comparatively extensive fall in the actual and relative mortality from tuberculosis in Colorado confined to cases of imported disease. This steady reduction

in mortality is not reflected in the group of cases in which the disease developed in Colorado. Second, as a result of invalidism from tuberculosis in the war forces, relatively large numbers of soldiers and sailors were sent to sanatoria in Colorado, and the mortality therefrom mounted to a figure which, associated with the epidemic of influenza, modified greatly the curve representing mortality from tuberculosis in Colorado.

THE RELATION BETWEEN THE MORTALITY RATE OF IMMIGRANTS AND THEIR LENGTH OF RESIDENCE IN COLORADO

We now come to a theme which, taken in connection with the influence of sex on the mortality rate, to be discussed later, appears to explain completely the eccentricities of the curves representing the mortalities from tuberculosis in Colorado.

It has been noted, charts 3 and 4, that the features of curves representing mortality from tuberculosis in Colorado are determined by the great numerical preponderance of deaths in cases in which disease had developed outside the State and in persons who had presumably immigrated in search of health. On chart 3 it is seen that the death rate from tuberculosis developed in Colorado is not only very low, but is represented by a tracing which takes a nearly horizontal course, falling slightly in the period of thirteen years, and is practically unaffected by the influence of the influenza epidemic which had such an extraordinary effect on the curve representing mortality in the subjects of imported tuberculosis.

Obviously, deductions as to the influence of environment on the mortality rates of a chronic disease like tuberculosis must take into account the period of residence of the invalid within that environment before the fatal termination of the disease.

A careful review of our records, with reference to "length of residence" of decedents in Colorado before death, revealed an astonishing situation. The subjects in question all developed their disease outside Colorado and were nearly all of adult age. Their deaths were classified in two groups: the first listed those deaths which occurred within three months of the arrival of the invalids in Colorado; the second included those deaths occurring after more than three months, but within one year, of residence in Colorado. It was found that a considerable proportion of the deaths occurred within a day or two of the invalids' arrival in the State. Careful consideration of the effect on the statistical returns,

in this particular, of the adoption of the new form of death certificate during the last two years of our study indicates that no material error was introduced into our results.

The total number of tuberculous invalids who died within three months of their arrival in Colorado during the thirteen years, 1908 to 1920, was 4,848; occurring after three months and within one year of arrival, the deaths numbered 4,691. The total of 9,539 gives an average of 733.8 per annum, of tuberculous invalids who died within a year after coming to Colorado for their health, 50.8 per cent of whom died within the first three months. Otherwise, our records seem to show that the number of tuberculous invalids who die within one year of their arrival in Colorado constitutes 40.4 per cent of the total mortality from tuberculosis in this State.

The average annual mortality from tuberculosis in Colorado for the thirteen years under investigation was 1,816 deaths; excluding the annual average of 733.8 deaths occurring within one year, the remainder is 1,082.2. The average population of Colorado for the same period was 873,082. We therefore find that, excluding deaths from tuberculosis which occur within one year of sojourn of newcomers in the State, the death rate for tuberculosis in Colorado falls to 123.9 per 100,000 of the population, as contrasted with the ratio of table 2 of 208.9 for Colorado and 147.5 for the Registration Area. In the last three lines of table 2 are represented the tuberculosis death rates per 100,000 of the population, according to the place of development of the disease. The rate for tuberculosis developed in Colorado is not affected by the above considerations. Recasting the averages of the last three lines of table 2 in their proper proportions, on the basis of a total death rate of 123.9 per 100,000 population, we find the following:

Deaths from tuberculosis in Colorado per 100,000 population	Disease developed outside Colorado....	67.6
	Disease developed inside Colorado.....	30.8
	Disease developed in unknown place...	25.5

As the stimulus to our work was the desire to add to the fund of medical climatology, we may venture to emphasize the protest, so often made in vain by those engaged in the social service aspects of tuberculosis in Colorado, against the practice, common alike to individuals and organizations throughout the more populous areas of the country, of sending away from home invalids so far advanced in disease as our figures prove.

When it is considered, in addition, that probably a large majority of the invalids in question are indigent and quite unable to secure that care and sustenance which medical experience has demonstrated to be of fundamental importance in the treatment of active tuberculosis, we are confronted with a situation unworthy of civilization.

THE INFLUENCE OF SEX ON MORTALITY FROM TUBERCULOSIS

Mortality rates from tuberculosis as modified by sex suggest some interesting topics for investigation. The exceptional composition of the population of Colorado is well illustrated by the fact that whereas, throughout the Registration Area there is but a slight numerical preponderance of males over females, the relative excess of males in Colorado is much greater. According to the U. S. Census, there were in Colorado for every 100 females in 1900, 120.9 males; in 1910, 116.9 males; and in 1920, 110.3 males.

Our figures seemed to show that with this preponderance in the male population there was still a considerable excess of mortality from tuberculosis among the male residents of Colorado. Table 3 presents the data from a definite investigation of these relations. We are indebted to Dr. M. H. Ames of the U. S. Navy for determination of the population of the Registration Area, according to sex, for the census years 1910 and 1920, since these are not given directly in the *Mortality Reports*. From these figures we estimated, by the arithmetical method, when necessary, the numbers of the male population for individual years, making the midyear period our basis of calculation. The male population was determined in the same way, though in the figures for Colorado a minor error is involved, dependent on the diminishing ratio of males. We could not locate in the *Census Mortality Statistics* the total number of male deaths in Colorado for the years 1912 and 1913, and were accordingly obliged to make estimates.

For the thirteen years under consideration the percentage of males in the population of the Registration Area varied, according to our estimates, from 50.8 in 1920 to 51.5 in 1908. In Colorado the percentage of males to the whole population appeared to vary in waves, from a minimum of 52.1 in 1912 to 52.4 in 1920 and 54.2 in 1908.

In the first two lines of table 3 is given the percentage ratio of male deaths to all deaths from tuberculosis in the Registration Area and in Colorado, respectively. In the third and fourth lines the same ratios are presented, except that the total mortality from all causes is sub-

stituted for that from tuberculosis. It will be seen in the fifth and sixth lines that there is a decided relative excess in the male deaths from tuberculosis in Colorado as compared with the Registration Area.

The same result, obtained in another way, has been represented graphically in chart 6. The percentage of males in the Registration Area and in Colorado, respectively, subtracted from the ratio percentage of male deaths to all deaths from tuberculosis in the corresponding populations, leaves a remainder that is much greater for Colorado than for the Registration Area. In the last two lines of table 3 it is seen that the excessive percentage of male deaths from tuberculosis in Colorado is an attribute of that fraction of the decedents whose disease was developed outside the State.

On chart 5 is represented graphically the actual number of deaths from all forms of tuberculosis in males and females, in separate tracings.

The pair of tracings designated as *A* represents mortalities for the whole thirteen years, 1908 to 1920. In *B* the tracings include only the ten years, 1908 to 1917, preceding the great epidemic. The tracings *C* repeat the conditions of *A* and are drawn to the same scale, but they represent only deaths due to tuberculous disease developed within Colorado.

The outstanding feature of tracings *A* and *B* is in the enormous excess in male deaths which is exhibited. The epidemic had little effect on the form of the curves, though during the three epidemic years there was a slight relative increase in the number of male deaths (9.3 per cent) as compared with female deaths (5.9 per cent). In the tracings marked *C*, representing mortalities from tuberculosis developed in Colorado, we fail to find the exaggerated preponderance of male mortality noted in *A* and *B*. An explanation in the striking difference between the curves will be offered presently.

Direct the attention to the first two decades of life represented in the tracings *A* and *B*. It is seen, namely, that up to two years of age there is a slight preponderance in the number of male deaths, perhaps accounted for by the excess of males in the population. But in the second decade, up to the twentieth year, there is an absolute excess of the number of deaths in females as compared with males. In the tracings *C*, representing mortalities from tuberculosis developed in Colorado, it is seen that the excess in the death of females persists through two decades, from the tenth to the thirtieth years of life. The explanation of these events will naturally be looked for in a failure of

vital "resistance" in the female during sexual maturation. Nevertheless, according to our data, there was in Colorado an actual excess of deaths from tuberculosis in females during the period from 3 to 9 years of age, inclusive, in the years 1910, 1912, 1915, and 1917 to 1920, inclusive. These facts illustrate in a small way a truth which the mortality statistics of the Census Bureau represent in a large one.

The compilations of the Census according to age and sex of the decedents, show constantly an excess of female deaths at certain ages, involving specifically the era of pubescence, but frequently manifested also before the age of five years. This interesting fact is illustrated, not only in the statistics of tuberculous disease, but is more or less in evidence in general respiratory disease, and is a veritable signpost in acute nephritis, Bright's disease and diseases of the circulatory system.

This interesting topic deserves special investigation; it must occupy a section of biometric literature which is unfamiliar to us. Here it concerns us chiefly as throwing light upon the peculiar course of the sex mortality curves from tuberculosis, constructed from the statistics for Colorado. It may be reiterated that the sex mortality curves representing deaths from tuberculous disease which developed in Colorado appear to represent sex mortality relations, as they exist throughout the Registration Area, much more closely than do the tracings depicting the variations of sex mortality of tuberculous subjects imported into Colorado; and our present endeavor is to explain these statistics.

Assembled on the same page (table 6) our records of death according to age and segregated according to locality in which the tuberculous disease developed, it is interesting to observe that in those deaths due to disease developed outside Colorado there is a sudden and very great numerical augmentation as we pass from the second to the third decade of life, and that the great majority of these deaths involve the male sex. When the same decade, beginning with the twentieth year of life, is considered in the smaller group of cases in which disease developed in Colorado, there is a relatively smaller increase of mortality in the third as compared with the second decade, and the relative number of male and female deaths is about equal; more commonly the female deaths are decidedly in excess. This, again, is just such a relation as seems to exist throughout the Registration Area.

THE INCIDENCE OF MULTIPLE TUBERCULOUS LESIONS

We have hitherto considered the sex influence in mortality from tuberculsois as manifested by the dominant form of the disease, in the

great majority of cases the pulmonary form, which is recorded on the death certificate as the *Cause of Death.* As will be seen in table 1 our compilation took note of the incidence of tuberculous *complications* of the principal disease.

We are interested to find, and have graphically depicted on chart 4, that there was a constant excess in the percentage of deaths in cases presenting multiple tuberculous lesions in the group of decedents whose disease developed outside of Colorado, compared with that of those whose disease developed within the State.

Our records show that during the thirteen years under consideration, in deaths from tuberculosis developed outside the State there were tuberculous complications in 1,589 cases, of which 1,131 or 71.1 per cent were males; the yearly average ratio of fatal male to female cases was 87 to 35.2. In deaths from cases developed within Colorado there were tuberculous complications in 159, of which 60 or 62.3 per cent were males. The yearly average of males to females was 7.6 to 4.6.

The tuberculous complications which have just been reviewed include the whole range of secondary tuberculous infections. Only one of these has received specific investigation, namely, tuberculous meningitis.

CAUSE OF THE HIGHER DEATH RATE IN MALES

It should now be plain that there is in Colorado, for some reason, a great relative augmentation of the death rate from tuberculous disease in males, and characterizing the more active periods of adult life. This disproportion is confined to the class of cases developed outside Colorado but dying after entering the State. It is very much less in evidence in the group of cases in which disease developed in Colorado or in the mortality manifests of the Registration Area. The relative excess of male deaths referred to in the group of cases imported into Colorado involves not only those considered from the viewpoint of the primary tuberculous disease but equally its secondary manifestations. That is, whatever causes the greater incidence in the rate of male mortality also, probably, during life rendered the decedent more susceptible to diverse organic implantation of his infection.

We are, then, confronted with a clear cut problem, to give the reason for *the high relative mortality rate in the male subjects of tuberculosis who immigrate to Colorado.* It has already been shown that an astonishing proportion of tuberculous health seekers who enter Colorado are so far

advanced in disease that they succumb within one year (many within a few days) of their arrival. Subtracting this moiety of decedents, who were obviously doomed under any conditions, there is a very substantial reduction in the excessive death rate ascribed to Colorado (from 208.9 to 123.9 per 100,000).

Now it is incredible that all the invalids severely afflicted with tuberculosis should have succumbed within one year. Many must have held to life, some to add to the mortality list of tuberculosis later, some to gain the goal of their desires and recover health. In short, excluding the large class of necessarily fatal cases, there is included in the horde of health seekers a still larger one in which the course of the tuberculous disease is determined by conditions wholly within human control.

The metabolism of any living organism is subject to two different phases of experimental procedure which may be distinguished in terms of nerve physiology, according as it excites the afferent and centripetal or the efferent and centrifugal nervous systems. To the former class of stimuli belong essentially all the influences of environment; which, concretely stated, includes climate. The second class of stimuli comes from within and consists in efferent nerve discharges, largely made up of motor impulses, and results for the individual in what we know as exercise. Now practically all we know of the rational treatment of tuberculosis may be included within these two agencies. Up to within a few years past environment and climate were the chief remedial agencies of the phthisiologist. But in recent times we have learned that the other phase of treatment is still more important. It consists in behavior which, reduced to its crudest term, we are accustomed to designate simply as muscular exercise.

It will be admitted that the metabolism of the subject with active tuberculosis is specifically sensitive to effort, the output of energy on the part of the individual. Clinical experience everywhere agrees that for the invalid with active tuberculosis *overexercise* is deadly. Opinions differ only in the placement of the line dividing deleterious from salutary exercise for, as has been said before, *Rest saves life, Exercise makes well.*

The authors of this report advance the view that the excess in the mortality rate of the tuberculous immigrants in Colorado is due, generally, to overexertion of unacclimatized individuals at a high altitude above sea level,—an environment which specifically stimulates to exercise and at the same time renders reaction to it more acute.

It will be noted that the figures representing sex mortality from tuberculosis developed in Colorado do not show the augmentation which we ascribe to lack of acclimatization. If the foregoing postulate is correct we should expect that the deleterious effect of overexercise in tuberculous newcomers to Colorado would be specifically manifested in their male contingent, for it is the male who is especially prone to try his powers, seek adventure and at times lapse into dissipation.

It is apparently a common injunction of his eastern mentor, to "take plenty of exercise in the open air and keep away from doctors," that guides the man with tuberculosis who is directed to Colorado.

It has been shown that when the mortality rate for Colorado has been corrected by the exclusion of cases dying within one year of their sojourn in the State the death rate from tuberculosis falls to 123.9 per 100,000. Subtracting the moiety 30.8 per 100,000, of deaths due to tuberculosis developed in Colorado, there remains a death rate of 93.1 per 100,000 of a population, a large part, if not the majority, of which is composed of individuals who have or have had tuberculosis. Such a situation is strong evidence of the salutary influence of climate on the course of tuberculosis.

INFLUENCE OF THE EPIDEMIC OF INFLUENZA

It still remains to account for the extraordinary increase in mortality in the subjects of imported tuberculosis which was manifested during the epidemic of influenza, extending from late in 1917 and continuing through 1920. This far exceeds the rate increase in tuberculosis mortality for the same period manifested in the general Registration Area or in the subjects of the disease developed in Colorado itself.

It should be noted that in our compilation of statistical data every notably tuberculous case dying in Colorado was, as far as possible, included.

The complicating nontuberculous disease must have often been the direct cause of death. The epidemic of influenza introduced an extraordinary excess of respiratory complications as determining mortality among the tuberculous. Given in round numbers, for the ten years 1908 to 1917 deaths from tuberculosis complicated with influenza and influenza-pneumonia formed an average of 0.83 per cent of the total mortality from tuberculosis, while the complication, pneumonia, occurred in 3.1 per cent of the deaths. But in the three years from 1918 to 1920 influenza and influenza-pneumonia were reported in 6 per cent

of the deaths in the tuberculous and pneumonia in 3.3 per cent additional cases of the total mortality. We can only conclude that the same internal conditions which lead to dissolution of the tuberculous immigrant from his primary disease produces in him a lowered resistance against the acute influenzal infection.

Comparing the relative mortality from tuberculosis in males to the total mortality from the same disease, for the four years 1913 to 1916, with the similar ratio for the epidemic years, 1917 to 1920, it is surprising to find a decided relative excess in the percentage of male deaths in the preëpidemic period, namely, 66.9 per cent for the years 1913 to 1916, as compared with 65.7 per cent for the period 1917 to 1920.

The thoughtful reader must have observed that a certain source of error, which we have not been able to eliminate, is involved in all our conclusions regarding the higher death rate from tuberculosis in the immigrant as compared with the indigenous population of Colorado, and especially as to the higher incidence of mortality in the male sex. We should know, to make our fundamental statistics complete, the relative proportions of the imported and the indigenous populations of Colorado and also the respective numbers of male and female invalids with tuberculosis who enter the State each year. It is hoped that these lacking data will be supplied by future investigation.

DEATHS FROM TUBERCULOSIS AMONG PERSONS BORN IN COLORADO

Our mortality data include the deaths of 1,206 persons who not only developed their tuberculous disease in Colorado, but were born and, in many instances, are recorded as having spent their lives in the State. In table 4 is given the sum of these deaths for thirteen years, distributed according to age and sex of decedents. It is seen that 535 deaths occurred during the first decade of life and 1,671 later. The great majority of deaths in the first decade were due to meningitis. There are 589 deaths in males and 617 in females. Already in the second decade there is a preponderance of mortality in females, which reaches its maximum ratio in the second and third decades and continues until the fifth. It is worth noting that the records show a large proportion of deaths in married females of early middle age. The average number of deaths per year was nearly 92.8; this is 34.6 per cent of the yearly average of deaths from tuberculosis developed in Colorado (268) and 5.1 per cent of the yearly average of total deaths from tuberculosis in the State.

MINERS' CONSUMPTION

Care was taken through correspondence with physicians, when necessary, to include in our tables only such victims of pneumokoniosis as had succumbed to the implantation of tuberculosis. The subjects were, of course, all of the male sex. They represented a total of 429 deaths. In the tracing at the bottom of chart 5 the mortality from this affection is seen to begin with the second decade of life and to reach its maximum in the fifth decade. The curve would indicate that tuberculosis has an increasing mortality rate in miners with advancing years.

SUMMARY

A study is presented of the statistics of mortality from tuberculosis in Colorado for the thirteen years 1908 to 1920.

A total of 23,608 deaths from tuberculosis is recorded, an average of 1,816 per year.

Deaths in cases of tuberculosis developed outside Colorado numbered 17,080, an average of 1,314.2 per year.

Deaths in cases of tuberculosis developed within Colorado numbered 3,484, an average of 268.0 per year.

Deaths in cases with place of development unknown numbered 3,044, an average of 234.2 per year.

The ratio of deaths in cases developed outside to those developed inside Colorado is as 83.1 to 16.9.

Subtracting from the Colorado mortality deaths due to tuberculous miners' consumption and tuberculous meningitis in the first decade of life, the proportion of deaths from tuberculosis developed in Colorado is reduced by about three units.

The death rate from tuberculosis for the thirteen years throughout the Registration Area averaged 147.5 per 100,000 population.

The death rate from tuberculosis in Colorado for the thirteen years averaged 208.9 per 100,000 population.

The death rate for Colorado, analyzed according to place where disease was developed, shows: developed outside Colorado, 150.5: developed inside Colorado, 30.8: place of development unknown, 27.5.

More than 40 per cent of deaths from tuberculosis occurring each year in Colorado are in newcomers who have sojourned in the State but one year or less; and of these 9,539 deaths 50.8 per cent occurred within the first three months of residence. Subtracting from the total mortality from tuberculosis the deaths occurring within one year of the decedents' residence in the State, the Colorado mortality from tuber-

culosis falls to 123.9 per 100,000 population; and, recasting the rates per 100,000 according to the place of development of the disease, we find that deaths from tuberculosis developed outside the State numbered 67.6 per 100,000 population; developed in the State, 30.8; place of development unknown, 25.5.

Tracings representing the course of mortality in the disease developed within Colorado are much more nearly parallel to those drawn to show similar conditions in the Registration Area than are tracings depicting Colorado deaths from tuberculous disease developed outside the State.

In the Registration Area males form an average of 51.1 per cent of the population.

In Colorado the excess of males is increased to 52.4 per cent.

There is proportionally a much higher death rate of males in Colorado than in the Registration Area. The percentage excess of male deaths in the two areas is as 6.7 to 1.7.

It is shown that the abnormal excess of male deaths is a feature confined to the immigrant tuberculous population of Colorado. A considerable but unknown proportion of this excess in male deaths may possibly be accounted for by excess of males among immigrant invalids.

Secondary or multiple tuberculous lesions are much more common in cases of disease developed outside than within Colorado; of the former, 9.4 per cent, and, in the latter, 4.6 per cent showed multiple lesions.

In spite of the excess of males in the population there is an absolute preponderance in the deaths from tuberculosis of females in the periods from the ninth to the twenty-ninth year in disease originating in Colorado and from the ninth to the sixteenth year in imported cases.

Our data justify the division of tuberculous meningitis into two clinical groups, as primary and secondary. The former is included chiefly within the first five years of life and the latter reaches its maximum at the twenty-fifth year.

Cases of pneumokoniosis succumbing to tuberculosis appear first about the sixteenth year of life, reach a maximum at the forty-sixth year and then decline; in actual number, 415 died in Colorado.

We have collected 1,206 cases of death from tuberculosis in which the decedents were born within the State. This is an average of 92.8 deaths per year and forms 34.6 per cent of the mortality in indigenous cases.

CONCLUSIONS

The results of this research emphasize the fundamental acquisitions of clinical experience in relation to the effects of environment and be-

havior on the course of tuberculosis. An important corollary to its teachings is the need of reform in the social management of advanced cases of disease.

The large number of deaths among tuberculous invalids within three months of their advent in Colorado is sufficient evidence of the hopeless condition of many of these invalids when sent from home. The lack of pecuniary resources makes too often impossible that kind of care which is necessary to successful combat with the disease.

We may plausibly postulate that these early deaths, particularly the fact that an excessive proportion of the male sex succumbs, are due for the most part to *overexercise*, more or less united with privations and worry, on the part of immigrants who have active disease and who do not allow themselves a period of rest for acclimatization.

When the excessive mortality rate for tuberculosis in Colorado is corrected by the subtraction of deaths occurring within the first year of residence, it is reduced to 123.9 per 100,000 population, as compared with 147.5 per 100,000 for the Registration Area. Subtracting again the death rate from tuberculosis developed in Colorado, 30.8 per 100,000 population, we have a remainder mortality rate from tuberculosis of 93.1 per 100,000 of a population a large proportion, if not most, of whose members have or have had tuberculosis. When it is considered that this group contains all the leftovers from the mass of invalids who have survived their first year's residence, we can only conclude that climate may offer an environment peculiarly beneficent to the needs of most tuberculous patients.

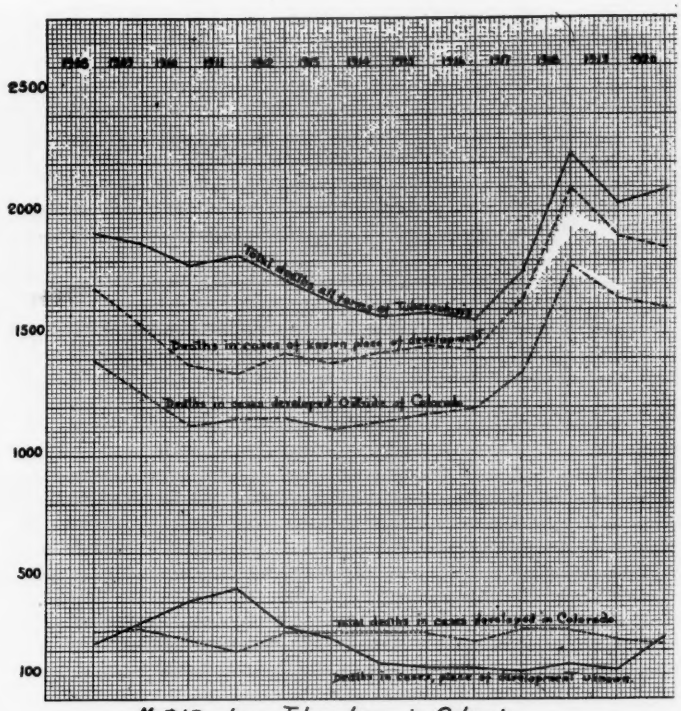
It is a great pleasure to acknowledge our indebtedness to Professor Raymond Pearl, of Johns Hopkins University, who at the outset of our undertaking reviewed and advised as to our plans of work.

Mr. G. H. Beaudry, Actuary for the Capitol Life Insurance Company of Denver, has given us unstinted aid in the more technical phases of our statistical work.

We are also indebted for advice, through Dr. L. K. Frankel, from the Statistician of the Metropolitan Life Insurance Company of New York.

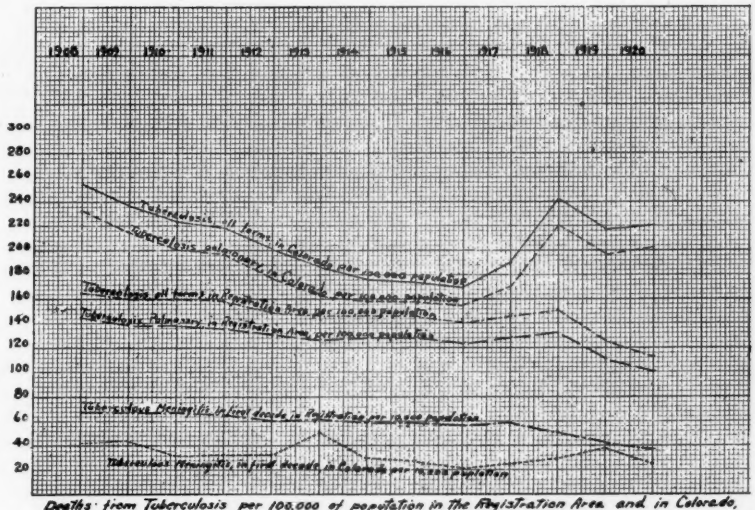
Dr. H. J. Corper, Director of the Research Department of the National Jewish Hospital for Consumptives at Denver, has revised to their advantage our tables and charts and made free the resources of his draughting department.

For the Committee,
HENRY SEWALL, *Chairman.*



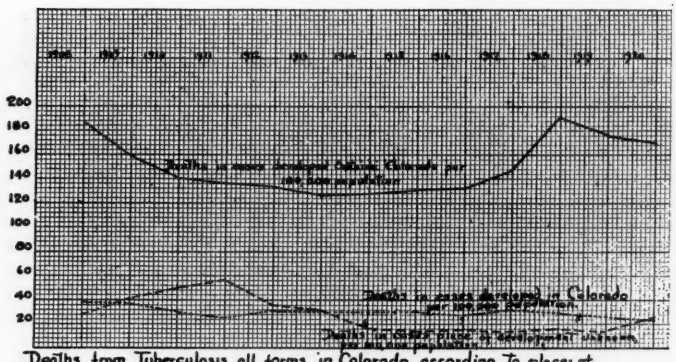
Mortality from Tuberculosis in Colorado.

CHART 1



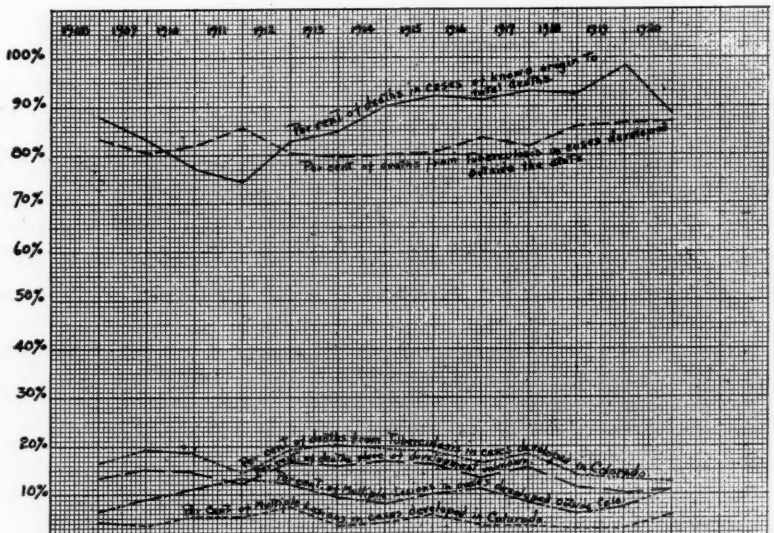
Deaths from Tuberculosis per 100,000 of population in the Registration Area and in Colorado, and from Tuberculous Meningitis in first decade of life per 10,000 population.

CHART 2



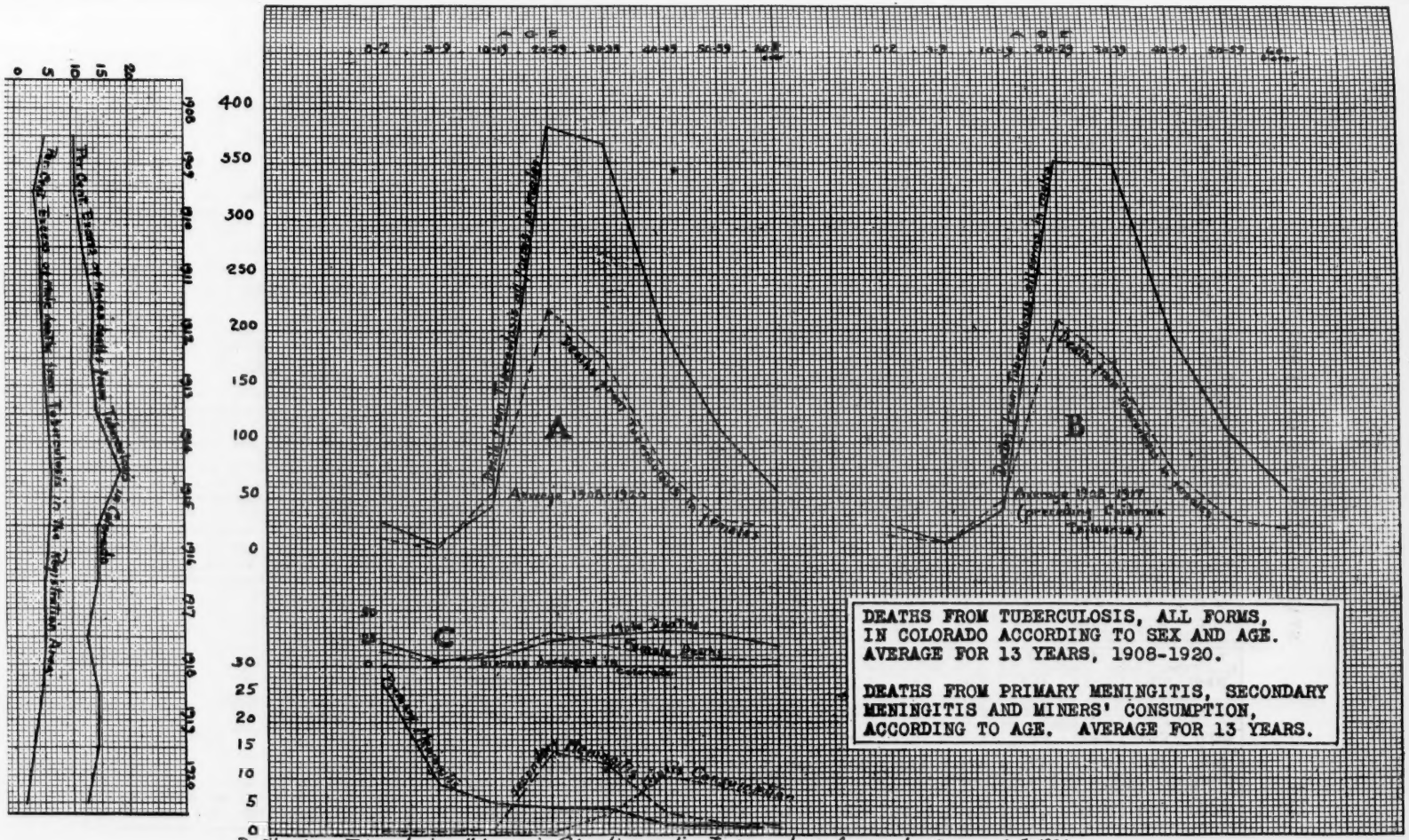
Deaths from Tuberculosis, all forms, in Colorado according to place of development, per 100,000 of population.

CHART 3



Per Cent. of deaths in cases of known origin to whole number of deaths from Tuberculosis in Colorado.
Per Cent. of deaths from Tuberculosis in Colorado in Cases developed Outside and In the State, and Per Cent. of deaths from Multiple Tuberculous Lesions in Cases developed Outside and In the State.

CHART 4


A
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C
DEATHS FROM TUBERCULOSIS, ALL FORMS, IN COLORADO ACCORDING TO SEX AND AGE. AVERAGE FOR 13 YEARS, 1908-1920.
DEATHS FROM PRIMARY MENINGITIS, SECONDARY MENINGITIS AND MINERS' CONSUMPTION, ACCORDING TO AGE. AVERAGE FOR 13 YEARS.

Deaths from Tuberculosis, all forms, in Colorado according to sex and age. Average for 13 years, 1908-1920.
Deaths from Primary Meningitis, Secondary Meningitis and Miners' Consumption according to Age. Average for 13 years

CHART 5

CHART 6
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TABLE 1

DATA FROM THE TUBERCULOSIS MORTALITY IN COLORADO FOR THE YEARS 1908-1920			1908	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920
			M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F
Number of deaths from Tuberculosis	TOTAL		1244 665 1909	1216 653 1869	1153 641 1794	1206 613 1819	1132 595 1727	1082 558 1640	1100 478 1578	1047 537 1584	1033 531 1564	1142 619 1761	1490 748 2238	1355 677 2032	1383 710 2093
	Developed Outside Colorado		918 479 1397	817 438 1255	729 405 1134	764 387 1151	750 397 1147	735 376 1111	817 323 1140	780 386 1166	790 403 1193	888 455 1343	1204 578 1782	1109 544 1653	1049 559 1608
	Developed In Colorado		184 30 284	179 116 295	161 94 255	127 74 201	169 112 281	185 96 281	184 100 284	144 117 283	140 93 233	181 117 298	184 110 296	144 109 253	138 102 240
	Developed In Known Place		1104 577 1681	994 556 1550	900 489 1389	853 499 1352	919 519 1428	918 474 1392	1001 423 1424	954 495 1449	936 490 1426	1049 592 1641	1390 688 2078	1273 633 1906	1167 681 1848
	Developed In Unknown Place		140 88 228	220 99 319	253 152 405	315 152 467	213 86 299	164 84 248	99 55 154	93 42 135	103 35 138	93 27 120	100 60 160	82 44 126	166 78 244
Percentage of Deaths from Known Origin	Developed Outside Colorado		84 29 83.1	85 28 80.9	83 28 81.6	87 26 85.2	85 27 80.4	85 27 79.9	87 23 80.1	84 26 80.5	85 29 83.7	85 27 81.9	85 28 85.8	86 29 86.8	87 30 87.1
	Developed In Colorado		11 6 16.9	11 7 19.1	11 7 18.4	10 5 14.8	11 8 19.6	14 6 20.1	12 8 19.9	11 8 19.5	9 7 16.3	11 7 18.1	9 5 14.2	8 5 13.2	8 5 12.9
Less Miners Consumption and Primary Meningitis in first decade of life			13.8	15	14	12.3	16.5	16	17.2	16.7	13.9	15.8	11.5	10.4	11
Number of deaths in cases having Multiple Tuberculous Lesions	Developed Outside Colorado		68 8% 84	78 9% 114	92 11% 123	104 12% 142	96 12% 136	86 10% 109	74 9% 97	82 10% 120	95 11% 133	73 9% 116	84 6% 109	87 7% 125	135 10% 189
	Developed In Colorado		10 8% 14	7 6% 11	7 6% 14	8 8% 11	12 9% 21	9 6% 10	9 9% 12	6 6% 17	6 4% 9	7 4% 11	8 5% 10	5 6% 8	9 6% 14
Total No. of Deaths in which Tuberculosis was associated with other diseases			257	262	267	215	248	215	286	227	204	282	384	307	436
Number of Deaths in cases with the four principal complicating diseases	Influenza & Influenza-Pneumonia		13 1%	19 1%	19 1%	16 1%	12 1%	4 .03%	11 1%	14 1%	14 1%	23 1%	178 8%	82 4%	124 6%
	Pneumonia		60 3%	49 2.6%	46 3%	49 3%	49 3%	61 4%	53 3%	49 3%	47 3%	57 3%	82 4%	66 3%	88 4%
	Nephritis		21 1%	40 2%	47 3%	34 2%	48 3%	24 1%	24 2%	39 2%	21 1%	58 3%	21 1%	18 1%	51 2%
	Cardiac Disease		36 2%	51 3%	46 3%	40 2%	44 3%	46 3%	45 3%	41 3%	52 3%	51 2%	30 1%	47 2%	75 4%
FORM OF THE TUBERCULOUS DISEASE	PULMONARY	0	1340	1196	1088	1108	1088	1054	1101	1124	1123	1275	1710	1586	1551
		I	201	190	182	140	182	187	191	192	156	210	211	162	156
		U	204	298	344	395	250	204	121	107	116	96	140	108	209
	MINERS' CONSUMPTION	0	0	4	1	2	3	6	0	1	3	4	5	2	4
		I	21	26	25	8	15	10	11	15	15	16	28	18	11
		U	2	1	20	25	22	22	11	15	12	11	7	4	9
	MENINGITIS Primary	0	5	13	6	7	11	11	10	5	13	15	22	19	8
	MENINGITIS Secondary	0	23	17	27	24	30	25	21	43	37	24	25	54	44
	MENINGITIS Primary	I	35	41	29	29	41	48	40	37	27	34	36	44	34
	MENINGITIS Secondary	I	3	1	2	4	5	1	1	2	3	0	3	1	5
	MENINGITIS Primary	U	5	6	15	17	8	5	5	1	2	2	5	5	7
	MENINGITIS Secondary	U	0	6	3	7	1	3	0	0	3	4	0	0	3
	OTHER FORMS OF TUBERCULOSIS	0	52	42	39	34	45	40	29	36	54	49	45	46	45
		I	27	38	19	24	43	36	42	39	35	38	21	29	39
		U	17	14	26	30	19	17	17	12	8	11	8	9	19

"O"-Outside Colorado "I"-In Colorado "U"-Unknown

TABLE 2

YEARS		1908	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	AVERAGE
POPULATION OF REGISTRATION AREA		44,783,913	50,570,516	53,643,674	59,482,100	60,350,203	63,197,158	65,672,424	67,083,421	71,339,485	74,972,578	81,371,843	83,147,822	87,484,713	
POPULATION OF COLORADO		751,973	788,234	804,495	830,756	857,016	883,276	892,361	901,446	910,531	919,616	928,701	937,786	946,871	
DEATHS FROM TUBERCULOSIS, ALL FORMS, PER 100,000 POPULATION	IN REGISTRATION AREA	167.6	160.8	160.3	153.2	149.7	147.8	147.2	146.3	142.1	147.1	150.0	125.6	114.2	147.5
	IN COLORADO	253.8	237.9	222.9	218.9	201.5	185.6	176.8	175.7	171.7	191.4	240.9	216.6	221.4	208.9
DEATHS FROM PULMONARY TUBERCULOSIS PER 100,000 POPULATION	IN REGISTRATION AREA	145.5	139.3	139.7	138.2	130.0	127.9	128.2	128.2	124.3	129.4	135.2	111.3	100.8	128.9
	IN COLORADO	233.5	216.4	200.6	197.7	177.3	163.6	158.3	157.8	153.2	171.2	221.9	197.9	202.3	188.6
DEATHS FROM TUBERCULOUS MENINGITIS IN FIRST DECADE OF LIFE PER 100,000 POPULATION	IN REGISTRATION AREA	6.8	6.6	6.5	6.6	6.0	6.3	5.9	5.9	5.8	5.9	5.1	4.2	3.8	5.8
	IN CASES DEVELOPED IN COLORADO	4.2	4.4	3.2	3.1	3.5	4.8	3.0	2.8	2.1	2.5	3.1	3.8	2.6	3.3
DEATHS FROM TUBERCULOUS MENINGITIS IN FIRST DECADE OF LIFE IN COLORADO	PERCENTAGE OF DEATHS IN CASES DEVELOPED IN COLORADO	10.9	11.8	10.2	12.9	10.6	14.9	9.8	8.8	8.1	7.7	9.7	14.2	10.4	10.8
	PERCENTAGE OF ALL DEATHS FROM TUBERCULOSIS RECORDED IN COLO.	1.6	1.8	1.4	1.4	1.7	2.5	1.8	1.5	1.2	1.2	1.3	1.7	1.2	1.6
DEATHS IN COLORADO FROM ALL FORMS OF TUBERCULOSIS, ACCORDING TO PLACE OF DEVELOPMENT, PER 100,000 POPULATION	IN CASES DEVELOPED OUTSIDE OF COLORADO	165.7	159.2	140.9	138.5	133.8	125.8	127.8	129.3	131.2	146.0	191.9	176.2	169.8	150.5
	IN CASES DEVELOPED IN COLORADO	37.7	37.4	31.7	24.3	32.8	31.8	31.8	31.4	25.6	32.4	31.9	26.9	25.3	30.8
	IN CASES PLACE OF DEVELOPMENT UNKNOWN	30.3	40.4	50.3	56.2	34.9	28.8	17.2	14.9	15.2	13.0	17.2	13.4	25.6	27.5

TABLE 3

COMPARISON OF THE MORTALITY RATES FROM TUBERCULOSIS IN MALES IN THE REGISTRATION AREA AND IN COLORADO

	YEARS	1908	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	AVERAGE
TUBERCULOSIS: RATIO PER CENT OF MALE DEATHS TO ALL DEATHS	IN REGISTRATION AREA	56.8	55.3	56.1	55.8	56.4	57.0	57.5	57.1	56.7	56.5	55.7	54.0	52.4	55.9
	IN COLORADO	65.2	65.1	65.9	66.3	65.5	65.9	69.7	65.9	66.0	64.8	66.5	66.7	64.6	66.0
DEATHS FROM ALL CAUSES: RATIO PER CENT OF MALE DEATHS TO ALL DEATHS	IN REGISTRATION AREA	54.3	54.2	54.6	54.4	54.7	54.9	54.7	54.4	54.6	54.8	53.9	52.8	52.4	54.2
	IN COLORADO	59.8	60.9	61.2	60.6	60.3	59.9	60.2	58.9	58.2	59.0	58.3	57.5	56.6	59.3
PERCENTAGE EXCESS OF MALE DEATHS FROM TUBERCULOSIS	IN REGISTRATION AREA	2.5	1.1	1.5	1.4	1.7	2.1	2.8	2.7	2.1	1.7	1.8	1.2	0.0	1.7
	IN COLORADO	5.4	4.2	4.7	5.7	5.2	6.0	9.5	7.0	7.8	5.8	8.2	9.2	8.0	6.7
TUBERCULOSIS IN COLORADO: PERCENTAGE OF MALE DEATHS	DISEASE DEVELOPED OUTSIDE COLORADO	65.7	65.1	65.1	66.5	65.4	66.1	71.6	67.6	66.2	66.1	67.5	67.1	65.2	66.5
	DISEASE DEVELOPED IN COLORADO	65.4	60.6	63.1	63.1	60.1	65.1	64.7	58.6	60.1	60.7	62.7	64.8	57.5	62.0

TABLE 4

Number of deaths in Colorado from primary and secondary tuberculous meningitis according to the locality in which the primary tuberculous disease developed

	TUBERCULOUS MENINGITIS			
	Primary		Secondary	
	M	F	M	F
Out of Colorado	98 68%	46 32%	323 81.9%	71 18.1%
In Colorado	275 57.9%	200 42.1%	21 67.7%	10 32.3%
Unknown	57 67.8%	27 32.2%	24 77.4%	7 22.6%
Total deaths	430 61.1%	273 38.9%	368 80.7%	88 19.3%
	703		456	

TABLE 5

Deaths from tuberculosis developed in Colorado of persons born in Colorado, segregated according to age and sex

Age	0–2		3–9		10–19		20–29		30–39		40–49		50–59		60 AND OVER	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Total	231	169	74	61	71	114	111	179	60	64	34	22	6	5	2	3
Sum of male and female	400		135		185		290		124		56		11		5	

DEATHS FROM TUBERCULOSIS IN COLORADO FOR YEAR OF 1917 *Table 6*

	AGE	0 - 2		3 - 9		10 - 19		20 - 29		30 - 39		40 - 49		50 - 59		60 & over		TOTAL	
Disease Developed	SEX	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Out of Colorado	Pulmonary	2		1	1	18	40	290	188	298	126	149	46	66	18	25	12	844	431
	Miner's Con.							1		1				2				4	
	Meningeal	1	1	1	(1)		(1)	4(1)	2(2)	5(1)	(1)	(1)		1				12(7)	3(7)
	Other Forms		1		1	3(1)	4(3)	6(3)	5(4)	9(2)	5(4)	3(2)	5(3)	2(2)	(2)	5	(1)	28(24)	21(24)
	Total	3	2	2	2	21	44	301	195	313	131	147	51	71	18	30	12	886(7)	455(10)
In Colorado	Pulmonary	5	2	1		3	13	23	23	32	15	24	10	25	6	18	10	131	79
	Miner's Con.									4		6		1		5		16	
	Meningeal	8	9	3	3	2	3	2	1	2	1							17	17
	Other Forms	3	1	(1)	1	4(1)	3(1)	2(1)	8	2(1)	3(1)	(1)	3(1)	6	1		1(1)	17(7)	21(7)
	Total	16	12	4	4	9	19	27	32	40	19	30	13	32	7	23	11	181(9)	117(9)
Unknown	Pulmonary					4	2	12	3	11	11	13	9	6	8	9	8	55	41
	Miner's Con.									2		4		4		1		11	
	Meningeal					1		1(1)		(1)			(1)			(1)		2(3)	(1)
	Other Forms		1					1	1(1)	(1)		2	1	2	2		1(1)	5(3)	6(3)
	Total		1			5	2	14	4	13	11	19	10	12	10	10	9	73(3)	47(3)
Grand Total		19	15	6	6	35	65	342	231	366	161	196	74	115	35	63	32	1192(7)	619(10)

Diseases Complicated by Tuberculosis																															
	Influenza & Influ-Pneu.		1						5	3	6	2	2				2	2													
	Pneumonia	1	2			1	1	1	12	1	12	6	5	1	5	3	2	4	3	1	25	10	4	10	4	4					
	Nephritis						3		7	6	7	4	16	1	6	2			1		23	6		13	4	1					
	Empyema									1	3	1			1						3				2						
	Cardiac dis.					1			6	1	9	5	7	3	6	3	1	1			22	7	1	14	5	2					
	Asthma										1	1	2				1				4				1						
	Syphilis												1								1										
	Peritonitis	1							2		1		1								1	1									
	Appendicitis						1					1	1	1							1				3						
	Surg. Shock															1										1				
	Pregnancy								2						1									1	1						
	Abscess Lung										1										1										
	Bronchitis														1																
	Pleurisy					1			2					1	1	1					1	1	4	1							
	Cirrhosis									1											1										
	Abscess							3	1	4				1		2					7	3		1							
	Diabetes							1		1		2	1	3		1					5	2		1							
	Intestinal Ulcer							1	2					1								1			2						
	Measles					1			1															1	1						
	Dysentery								1		1			1							2										
	Appendicitis																				1										
	Tonsilitis		1							1														1	1					
	Cardiac Lues									1															1						
	Ulcer Stomach										1														1						
	TOTAL	4	4			3	8	42	32	49	22	43	11	27	13	16	6	3	4	3	43	5	3	30	9						

Total number tuberculosis cases 1761 Less complicated cases 282 = 1479

Number of deaths in which certificates of death were fully completed 1323

" " " " " " " not " 438

" " letters of inquiry as to lacking data 409

" " replies sufficiently indicating place of development of disease 275

Corrected number of satisfactory certificates 1323+275 = 1598

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